STUDIES ON N-FIXING AND P-SOLUBILIZING MICRO-ORGANISMS IN SOIL AND PLANTS

A

THESIS

Submitted to the

University of Allahabad

For the Degree of

DOCTOR OF PHILOSOPHY

In Agricultural Chemistry and Soil Science

By

Sanjay Kumar Singh

M.Sc. (Ag. Chemistry & Soil Science)

Under the Supervision of

Prof. M. M. Verma

SHEILA DHAR INSTITUTE OF SOIL SCIENCE

Department of Chemistry

University of Allahabad

Allahabad

2002

Enrollment No. 88 AU/498

UNIVERSITY OF ALLAHABAD
SHEILA DHAR INSTITUTE OF SOIL SCIENCE
5, LAJPAT RAI ROAD
ALLAHABAD 211 002 (U. P.) India

Dr. M. M. Verma
Professor
Agricultural Chemistry & Soil Science

Date: 02. 11. 02

CERTIFICATE OF ORIGINAL WORK

This is to certify that the thesis entitled "STUDIES ON N-FIXING AND P-SOLUBILISING MICRO-ORGANISMS IN SOIL AND PLANTS" submitted to the University of Allahabad, in fulfilment of the requirements for the degree of *DOCTOR OF PHILOSOPHY (AGRICULTURAL CHEMSTRY AND SOIL SCIENCE)*, is a record of bonafide research work carried out by *Shri Sanjay Kumar Singh*, under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

To the best of my knowledge, experimental observations and data presented in the thesis are genuine and original.

(M. M. Verma)
Supervisor

Acknowledgement

It gives me great pleasure to express my deep sense of gratitude to my Supervisor, Dr. M. M. Verma, Professor, Agricultural Chemistry and Soil Science & Director, Sheila Dhar Institute of Soil Sciences & Head of the Chemistry Department, University of Allahabad, under whose able and active guidance and supervision, the entire research work of this thesis was completed.

I take this opportunity to extend my sincerest thanks to Dr. A. K. Singh, Reader, Chemistry Department, University of Allahabad.

I am grateful to Dr. A. B. Singh, Scientist, I. I. V. R., Varanasi and Dr. Ajay Kumar, Research Associate, Allahabad Agricultural Institute – Deemed University, Allahabad, for their help in statistical analysis of data by computer.

I am thankful to **CORDET, IFFCO**, Phulpur Unit, Allahabad, for providing the facilities for analysis of soil and plant material.

I am grateful to Dr. Ravi Shankar Singh, Shri Jagat Narayan Singh and Shri Swatantra Bajpai, who helped me during the entire course of my research work.

I am also thankful to my brother in law Shri Brajesh Singh and sister Smt. Prema Singh, who gave me profound affection and co-operated me at every step towards my higher education.

I am thankful to Shri Chitranjan Sharma, Research Scholar, S. D. D. of Soil Science and Shri Rajkumar (Raju), Lab. Attendent for the miscellaneous help rendered to me during the preparation of the thesis.

Last but not the least, I gratefully remember the blessings of my beloved parents, Late (Smt.) Shanti Devi and Late (Shri) Ram Karan Singh, without which this thesis would not have seen the light of the day.

My thanks are also to Shri N. R. Bagchi for his sincere and untiring efforts in processing and printing of this thesis.

(Sanjay Kumar Singh)

Place : Allahabad
Date : 02.11.2002

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER – I

INTRODUCTION

INTRODUCTION

The term "biofertiliser" or more appropriately a "microbial inoculant" can be generally defined as preparations containing live or latent cell of efficient strains of nitrogen fixing, phosphate solubilising or cellulolytic microorganisms used for application to seed or composting areas with the objective of increasing the numbers of such microorganisms and accelerate those microbial processes which augment the availability of nutrients that can be easily assimilated by plant.

"Green Revolution" started and continued from 1960 to 1980 in India, which resulted in increased agricultural production. It was due to high yielding fertiliser responsive varieties of crops. Thus, the pathway to increased agricultural production is accompanied by an exponential increase in consumption of non-renewable sources of energy. In view of the fast fall of energy sources combined with their escalation costs. It is not unlikely that a future, for developing countries like India, energy can become a limiting factor for increasing agriculture production. It is therefore, essential that a strategy for integrated nutrient supply is evolved by using a judicious combination of chemical fertilisers, organic manures and biofertilisers.

Biofertilisers harness atmospheric nitrogen with help of specialised microorganisms which may be free living in soil or symbiotic with plant, recent reports indicate that these self perpetuating bodies can make significant contribution in productivity improvement in economically stricken countries like India.

The ratio between chemically fixed and biologically fixed nitrogen usually ranges between 1 : 4 to 1 : 25 and within biological fixation, the legume fixation is at least ½ of industrial fixation. The demand for chemically fixed nitrogen is bound to be on the increase and thus the nitrogen gap is also likely to be magnified. In developing countries, establishment of fertiliser plant is not only expensive but also time taking, the strategy for improving agricultural production in India should, therefore, take into account the inexpensive biological nitrogen fixation programme.

'Microbial inoculants' are carrier-based preparations containing beneficial microorganisms in a viable state, intended for seed or soil application. This is designed to improve soil fertility and plant growth by increasing the number of desired microorganisms in plant rhizosphere. Following their success, a large number of inoculant industries are developing all over the world including India.

Types and Scope of Microbial Inoculants

A. Nitrogen fixers

1. Symbiotic

   - Rhizobium inoculants for Legumes

2. Non-Symbiotic

   for cereals, millets, vegetables

   a. Bacteria –

      i. Aerobic - Azotobacter
         Azomonas
         Azospirillum
         Mycobacterium

      ii. Anaerobic - Clostridium
         Chlorobium
         Chloromatium

      iii. Facultative anaerobic - Bacillus
         Enterobacter
         Eischerichia
         Rhodospirillum

b. Blue – green algae - Anabaena

Anabaenopsis

Nostoc

Tolypothrix

B. Phosphate solubilising microorganisms

C. Cellulolytic and lignolytic microorganisms

D. Sulphur dissolving bacteria

E. Azolla

In India, microbial Inoculants Azotobacterin, Rhizobacterin, Phosphobacterin, Algal-bacterin are being more worked out because of lack of inadequate N and P_2O_5 supply to plant. While possibility of P_2O_5 fixation from atmosphere is feeble, attention is being paid to make P_2O_5 available biologically (Phosphobacterin) from insoluble phosphate sources like rock phosphate and basic slag. it was estimated earlier i.e. during sixties, the cost of quantity of inoculant requires to boost N and P_2O_5 per hectare of land was only 35 P per hectare (now approx. Rs. 7.00 per hectare) for getting 10 to 30 percent increase of crop growth. The amount of the culture used per hectare of land varied between 5 g and 15 g depending upon the size of grain to be cultivated.

Azotobacterin:

The distribution of Azotobacter in different soil of the USSR has been extensively studied by soil plate method. It has been shown that a considerable number of colonies of Azotobacter develop on soil plates from cultivated and manured soil samples of the USSR in contrast of virgin meadow and forest soils where the number are low or negligible. Sporadic studies of distribution have also been made in India.

Azotobacter cells are not usually present on the rhizoplane (root surface) but are abundant in the rhizosphere (the soil immediately surrounding roots). The dominance of Azotobacter in the rhizosphere of plants has been consistently shown by several investigators, although some plants like wheat are known to harbour more anaerobic clostridia type of bacteria in their rhizosphere than aerobic Azotobacter types (**Subba Rao, 1994**).

Azospirillum:

Azospirillum, an associative micro-aerophillic Nitrogen fixer, commonly found in loose association with the roots of cereals and grasses, is of great interest. High nitrogen fixation capacity, low energy requirement and abundant establishment in the roots of cereals and tolerance to high soil temperature (30–40°C) are responsible for its

suitability under tropical conditions.

Azospirillum is mesophillic and reported in association of crops grown in acidic to alkaline pH range. Azospirilla are metabolically versatile and can grow vigorously in presence of nitrogenous compounds present in soil but as soon as the external nitrogen supply is exhausted the bacteria switch on to diazotrophy. The ability to fix nitrogen is unaffected by the presence of combined nitrogen sources (**Shukla and Kundu, 1986**) and may account for the beneficial response of Azospirillum inoculation in fields receiving mineral fertilizers. Use of Azospirillum inoculation under saline alkaline condition is possible because strains adapted to these stress conditions maintained high nitrogenase activity.

The mechanism of the bacterization resulting in yield increase with decrease or no increase in N concentration may be attributed to enhanced N fixation or increased N assimilation by plants. In vitro Azospirillum lipoferum produces siderophores when grown in iron-deficient medium. Production of siderophores by the bacterium may improve iron-nutrition.

Azospirillum inoculation has shown positive interaction with applied N in several cereals with an average response equivalent to 15 – 30 kg/ha of applied N (**Subba Rao *et al.*, 1982**). Dipping roots of rice seedlings in 2 percent solution of Azospirillum increased the yield

by 200 kg/ha over un-inoculated treatments (**Mahapatra and Sharma, 1988**). Beneficial effect of rice seedling root dippings in Azospirillum was also reported by many research workers (**Jeyram and Ramaiah, 1986** and **Kumar and Balasubramaniam, 1986**). Rice yield increase of 33 percent by **Kannaiyan *et al.* (1983)** and 28 percent under irrigated and 16 percent under rainfed condition by **Kumar and Balasubramaniam (1986)** were reported due to inoculation of rice seedling with Azospirillum.

Increase in yield due to seed inoculation was also reported in wheat. Application of fertilizers enhanced the benefit of inoculation with Azospirillum lipoferum in wheat (**Rai and Gaur, 1982**). Varietal difference in response to Azospirillum inoculation in wheat was observed by **Indu Bala and Kundu (1988)**. **Sawarkar and Goydani (1996)** from Chhindwara reported that the seed treatment of wheat with Azospirillum @ 5 g/kg seed could substitute 50 percent N amounting to 15 kg N/ha under rainfed conditions.

Rhizobium:

Rhizobium is one, which fixes the nitrogen symbiotically, i.e. harbouring one or other host plants. Rhizobium is a genus of aerobic, heterotrophic, non-spore forming soil bacteria, able to invade roots of leguminous plants and form nodules in which atmospheric nitrogen is fixed. The number of effective nodules and functioning of the nodular

tissue are enhanced by artificial seed inoculation with appropriate rhizobia. Approximately 14 million tonnes of nitrogen is fixed annually by legume on a global scale which is about half of the annual global output of 30 million tonnes of industrially fixed nitrogen. It reflects the importance of legumes in general and pulse crops in particular as viable sources of biological nitrogen crop production. The low yield of pulse in the country is also due to lack of effective strains of rhizobia in adequate number in soil (**Rao and Nagarajan, 1972** and **Rao, 1975**).

It is expected that after the inoculation the population of effective rhizobia near the root system will increase and enhance the degree of N_2 fixation. The fixed nitrogen, which occurs in protruded section of the host root (nodules), is gradually metabolised inside the host body. This helps in a better growth and yield of shoots, roots and ultimately grains.

Phosphorus solubilizing microorganisms:

Of the three major mineral elements, phosphorus plays both direct and indirect role in the metabolism of plant and micro-organism. Its major physiological role at certain essential steps in the accumulation and release of energy during cellular metabolism. Researchers over the years have revealed that phosphorus causes disturbances in one of more vital plant processes resulting visual

symptoms of disorders.

Several soil bacteria, particularly those belonging to the genera Pseudomonas and Bacillus, and fungi belonging to general Penicillium and Aspergillus possess the ability to bring insoluble phosphates of soil into soluble forms by secreting organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric and succinic acid. These acids lower the pH and bring about the dissolution of bound forms phosphate.

Recently emphasis has been placed on the possibility of greater utilisation of indigenously available rock phosphate resources by the action of phosphates solubilising microorganisms. In this connection, field experiments have been carried out in India using culture suspensions of B. polymyxa, B. Clrculans, P. Striata and Aspergillus awamori with and without super phosphate or rock phosphate on the yield of wheat and rice (Gaur *et al.*, 1980). The result demonstrated the significant increase in grain yield is possible when wheat was inoculated with P. Striata in the presence of rock phosphate at 100 kg P_2O_5/ha.

The efficiency of phosphatic fertilizers is very low (15 - 20 percent) in soil due to its fixation in soil. Besides, native soil phosphorus is usually unavailable to crop because of its low solubility. The introduction of efficient phosphate solubilisers in

rhizosphere has been found to increase the availability of phosphorus from both applied and native soil phosphorus. The phosphorus which get fixed in soil is made available to crops by the action of certain microorganisms which solubilise insoluble/fixed forms of microorganisms which readily taken up by the plants. Another factor that hinder ineffective utilisation of P in its low mobility in soil. Phosphate solubilising microorganisms (PSM) includes various bacterial, fungal and soluble forms. Species of Pseudomonas. Micrococcus, Bacillus, Flavobacterium, Penicillium, Fusariam, Sclerotium and Aspergillus are some of phosphate solubilisers. Among these, soil bacteria belonging to the genera Penicillium and Aspergillus are more common. The solubilisation effect of PSM is generally due to the production of organic acid by these organisms. They are also known to produce amino-acids, vitamins, growth promoting substances like indole acetic acid (IAA) and gibberelic acid (GA), which help in better growth of plants.

Inoculation of seedlings with phosphates solubilising organisms resulting in increased uptake of P and increased crop yield was first shown in 1948 by Gerretsen, since then beneficial influence of artificial inoculation with phosphate, dissolving organisms has been reported from different crops under diverse agro-climatic conditions.

On the basis of these findings and informations, the present

research work has been carried out in this thesis entitled "Studies on N-fixing and P-solubilising microorganisms in soil and plants with the following objectives:

1. To study the response of Mussoorie Rock Phosphate (MRP), Rhizobium, MRP and Azospirillum, MRP and phosphate soluble micro-organism (PSM) on plant height,Number of pods, yield, fresh weight and dry weight content of Pea (*Pisum sativum* L.) variety Azad P_1.

2. To study the response of MRP, Rhizobium, Azospirillum and PSM on nitrogen- phosphorus uptake in Pea.

3. To study the response of MRP and Azotobacter, MRP and PSM, MRP and Azospirillum on plant height, grain/straw yield and nitrogen phosphorus uptake in wheat (*Triticum aestivum*) variety Sonalika.

CHAPTER – II

REVIEW

OF

LITERATURE

REVIEW OF LITERATURE

In India systematic research on biofertilizer started with the first study of N. V. Joshi in 1920. This was followed by extensive research by Ganguli, Sakaria and Madhok on physiology of nodule bacteria and its inoculation for increasing crop yields, important milestones in production, development and promotion of biofertilizers in India and given in following lines:

Some milestones in research and promotion of Biofertilizer in India:

1920	First study on legume Rhizobium symbiosis by N. V. Joshi
1934	Earliest documented production of Rhizobium inoculant by M. R. Madhok .
1956	First commercial production of biofertilizer
1957	Study on solubilization of phosphate by microorganisms by Sen and Pal
1964	Spirit in demand of Biofertilizer for Soyabean, particularly in M.P.
1970	Scope for use of charcoal lignite and FYM as alternate carriers to peat reported by V. Iswaran
1977	Use of I S I mark for Rhizobium

1983 Setting up of National project on development and use of biofertilizers by Ministry of Agriculture, Government of India

1986 Setting up of National facility for BGA collection at I A R I, New Delhi, by the Department of Biotechnology, Government of India

In a green house trial with Pea (*Pisum sativum*) **Yousry *et al.* (1978)** reported that phosphobacterin inoculation increased plant dry matter by 10.9 percent when compared with uninoculated control. **Pikovskaya (1948)** obtained an increase in green matter yield in Oat plants due to inoculation of phosphobacteria. According to **Shestakova (1963)** aqueous formulation of phosphobacterin increased lime and pine weights at their intensive growth period by 20–25 percent and 16–19 percent, respectively. Phosphobacterin inoculation increased the growth and yield of Soyabean (**Arora, 1975**), Broad bean (**Taha *et al.*, 1969**), Maize (**Sharma and Singh, 1971**) and Chick pea (**Ahmed and Jha, 1977**).

Yield increases from the use of phosphobacterin ranged from 0 – 70 percent (**Smith *et al.*, 1961**). However, the average increase was about 10 percent. It was also found that yield of tomato grown in greenhouse with phosphobacterin inoculation increased upto 7.5 percent (**Smith and Allison, 1962**).

Gerretsen (1948) reported that CO_2 produced by microorganisms in the Rhizosphere seems to be an effective mechanism in increasing the availability of phosphorus and its uptake by plants. **Sperber (1957)** suggested that phosphorus is released in soil from inorganic compounds due to local accumulation of lactic acid and action of H_2S developed by microbial metabolism. These two biological products were found important in rendering insoluble phosphate into available form.

The phosphobacterin inoculation caused a decrease in soil pH due to the formation of organic acid (**Sundara Rao and Sinha, 1963**) supports the view that CO_2, H_2S and acids, the end products of microbial activities effect the solubilization of phosphates. **Kundu and Gaur (1981)** suggested that solubilization process is carried out by diverse physiological groups of microorganisms like bacteria, fungi and actinomycetes.

Gurumurthy and Sreenivasa (1996) observed that the fresh fruit weight, plant dry weight and shoot phosphorus concentration in chilli were increased with increase inoculum level of phosphates solubilizers upto 50 g/10 kg soil. Further increase in these parameters beyond 50 g inoculum level were found non-significant.

Yadav and Srivastava (1997) in a field trial on chickpea at Morena (M. P.) studied the response of phosphate solubilizers with

graded level of phosphorus (0, 20, 40, 60 kg P_2O_5/ha). They obtained the highest yield of 2.67 t/ha by giving 60 kg P/ha + PSB followed by 60 kg P alone. The uninoculated control yielded only 1.1 t/ha. Similarly, Srivastava *et al.* (1998) in a field trial on pea cv. Arkel at IARI, New Delhi, reported that application of 25.8 kg P, 0.5 kg Mo and seed inoculation with either R. legminosarum or with PSB or both resulted significant increase in nodulation, nitrogenous activity, vegetative growth and grain yield. However, dual inoculation yielded better response than single inoculation of bacterium.

Kumaran *et al.* (1998) in a field trial with Tomato at Coimbatore revealed that Azospirillum and phosphobacteria gave significant response in terms of growth and yield, plant height, branches / plant, mean fruit weight and number of fruits / plant were significantly improved with Azospirillum and phosphobacteria treatment.

Singh *et al.* (1999) studied the integration of organic manures, inorganic phosphates and phosphate solubilizers on growth and yield of potato cv. Kufri Megha and Kufri Jyoti at upper Shillong, Meghalaya. They reported that supplementing the field with FYM @ 20 t/ha and inoculation of PSB strain pseudomonas striata combined with 26 or 52 kg P/ha significantly enhanced the yield.

1. Isolation of Azotobacter (General Technique) and isolation by soil dilution-plating method:

Soil sample of 10 g is transferred in 100 ml. of sterilized water and serial dilution of the suspension are prepared by further dilution using sterile distilled water. Petriplates and pipettes are sterilized. Any one of the nitrogen free agar media specific for Azotobacter is prepared and poured into petriplates. One ml. aliquot of appropriate dilution are spread evenly over cooled and set agar medium in petriplates. The plates are incubated at 30°C in an incubator. After 3– 4 days of incubation, soft, milky hyaline and mucoid colonies of Azotobacter are formed an agar plates.

Direct isolation of Azotobacter has been practised by Russian scientists. Lumps of soil are spread out on a nitrogen free agar medium and the plates are incubated at 28°C. After three days of incubation, Azotobacter colonies develop on the agar plates.

Another method is to prepare a paste of soil in nitrogen free liquid medium. This paste is incorporated in a layer of washed charcoal placed in a petriplate. Soil is aerated by burying a glass tube in it. After incubation on the plates at 28°C for 3–4 days, small Azotobacter colonies, if present develop on the soil surface. This method is popularly known as soil plate method.

2. Identification of Azotobacter:

The bacterium is identified on the basis of cell morphology, shape, size, flagellation, cyst information, polysaccharide or gum production and pigment formation.

3. Classification of Azotobacter:

Recognised species of Azotobacter are – A. chroococcum, A.vinelanclii, A. beiterinckii, A.paspalli, A.macrocytogenea, A.insignis and A.agilis. The first species of Azotobacter is one of the most predominant one in Indian soils.

4. Maintenance and cultivation of Azotobacter:

Azotobacter isolates are maintained on nitrogen free agar medium and periodically sub-cultured. Large scale cultivation is done either in large flasks on rotatory shaker or in batch fermenters by aseptic method.

5. Carrier based inoculants of Azotobacter:

These are prepared in the same way as Rhizobium inoculant, carriers like powdered soil (peat), lignite or farm yard manure are neutralised with $CaCO_3$, autoclaved and mixed with broth culture of Azotobacter. The mixture after curing in trays for 2-5 days is packed in polythene bags.

6. Inoculation method:

A slurry of carrier based inoculant is made with minimum amount of water and seed are mixed with the slurry, dried in shade and sown; seedling dip (10–13 minute) in slurry is done for transplanted crops are planted immediately. For sugarcane etc., secondary inoculation with slurry near the root zone in early stages of plant growth are also recommended. The inoculants can also be mixed with gur of FYM and broadcast near the root zone.

7. Function and crop response to Azotobacter application:

From the field experiments with various Azotobacter inoculants on onion, wheat, rice, brinjal, tomato, cabbage, sugarcane, oat, barley, maize, potato indicate 0–30 percent increase in crop yield, Azotobacter spp. increases plant yield primarily by fixing molecular nitrogen in soil but it is also reported to synthesize auxins, vitamins, growth substances and anti fungal antibiotics which have beneficial effects on seed germination etc. works on these aspects have already been given earlier.

Rokade and Patel (1992) – important phosphate solubilizing organisms are pseudomonas striata, Bacillus polymyxa, Aspergillus awamori, Penicillium digitatum. P. solubilizing fungal population is generally found more in acid to neutral soils while the bacterial population in neutral to alkaline soils. These microorganisms can

grow on insoluble phosphatic sources such as tricalcium phosphate, ferric, aluminium and magnesium phosphate, rock phosphate and bone meal and convert them into soluble forms. A variety of mechanisms are ascribed in the solubilisation and mineralisation of insoluble and organic P sources, such as production of aliphatic, aromatic acids, phytases, phospholipases, etc. The efficiency/ performance of these microorganisms is affected by availability of a carbon source, P concentration, particle size of rock phosphate and other factors like pH, temperature and moisture.

Sundara Rao (1968) – Out of 37 field trials conducted in India during 1960s, 10 trials showed significant increase in yield of wheat, rice and maize. Field studies carried out under AICARP (1969 – 71) have indicated significant effect of inoculation with Bacillus megaterium and B. circulans in rice and wheat which was equivalent to the yield increase obtained with upto 50 kg P_2O_5.

Sharma and Singh (1970) and **Kundu and Gaur (1984)** – Beneficial effect of phosphorus biofertilizers in increasing grain yield and P uptake was also reported by many workers.

Datta *et al.* (1992) – Bacillus furmus along with rock phosphate and organic manure served as excellent biofertilizers which was superior to single super phosphate in acid soils.

Gaur (1988) – Soil inoculation of P.striata besides increasing grain yield of wheat and pea (*Pisum sativum* d.) showed residual effect by increasing the grain yield of Maize crop in alluvial soil at Delhi.

Tiwari *et al.* (1993) – For wheat in alkaline loamy soils, P.striata was more efficient than B.polymyxa and A.awamori.

Modak *et al.* (1994) – In Pigeonpea (*Cajanus cajan* L.Millsp.) wheat sequences there was no significant effect of seed inoculation with phosphobacteria on pigeonpea but wheat yield increased with seed inoculation of the preceding pigeonpea crop.

AICSMIP (1986–88): Seed inoculation with A.awamori increased the yield of finger millet by 23 percent and this in combination with Azotobacter chroococcum and Azospirillum brasilense further increased yield by 28.9 and 36.2 percent respectively.

AICSMIP (1988–91): A combination of superphosphate and rock phosphate on equal P basis along with seed inoculation with Agrobacterium radiobacter and A. awamori increased the productivity by 8–28 percent in finger millet, 3–6 percent in foxtail millet (*Setaria italica* L. Beauv.), 70 percent in kodo millet (*Paspalum scrobiculatum* L.), 21 percent in little millet (*Panicium sumatrense* Roth.) and 31 percent in barn yard millet (*Echinochloa crusgalli* Beauv.) compared to

application of P as super phosphate alone without seed inoculation.

Tilak (1991) – The microphos culture developed at I A R I, New Delhi, containing pseudomonas striata and Bacillus ploymyxa was tested in multilocation field trials with rice and wheat and results invariably showed the inoculation of seed and seedlings increased the grain yield both without P and with rock phosphate.

Gaur (1990) - There are substantial deposits of low grade rock phosphate in the country which can not be used for direct application in neutral to alkaline soils. Integrated use of suitable microbial cultures along with low grade rock phosphate can add about 30 kg P_2O_5/ha. To achieve this, it is imperative that efficient organisms adapted to local conditions should be isolated and congenial condition created for their establishment in the root regions by deserving better inoculation techniques and modifying the hostile soil environment. There is also need to intensify research efforts to identify areas and crops for which such artificial inoculations are likely to be more successful.

CROP RESPONSE:

Inoculation of seedlings with phosphates solubilizing organisms resulting in increased uptake of P and increased crop yield was first shown in 1948 by Gorretsen. Since then beneficial influence of

artificial inoculation with phosphate dissolving organisms has been reported from different crops under diverse agroclimatic condition in India. The microphos culture containing pseudomonas striata and Bacillus ploymyxa was tested in multilocational field trials with wheat, rice, chickpea, soyabean, lentil and potato. Results obtained showed that inoculation of seed and seedlings with PSB increased grain yield of crops. The use of cultures increased the efficiency of rock phosphate and super phosphate applied to neutral alkaline soils.

The role of phosphate solubilizing organisms in increasing crop yield is subjected to controversy due to inconsistent results of many places.

Of the total inoculation experiments conducted so far, a significant increase in yield of 5 to 10 percent has been observed only in about 25 percent experiments. Several factors such as carbon and nitrogen sources, rock phosphate concentration, particle size of rock phosphate, pH, temperature aeration, incubation period, etc. affect the efficiency of phosphate solubilizers. In general, the response to phosphobacterins was found to be positive only in soils with high organic matter content and low in available phosphorus.

Aspergillus (**Karaguishieva, 1967**): Azotobacter treatment resulted in lower incidence of certain fungal diseases (**Berezova *et al.*, 1938**) and higher percentage of healthy seedling (**Naumova, 1939**).

The incidence of potato scab was considerably reduced with Azotobacterin. **Lakshmi Kumari *et al.* (1972)** studied the interaction between Azotobacter spp. and fungi and found that Azotobacter produced weak antifungal antibiotics.

Ability of Azotobacter to produce growth regulating substances has also been reported. Extra cellular secretions of A.agilis and A.chroococcum stimulated the growth of some microorganisms (**Sandark, 1971**), culture supernatants of A.vinelandii and A.beijerinckii contained auxins, at least three gibberellin like substances and three cytokinin like substances. Root inoculation of tomato seedlings with these cultures-accelerated plant growth and increased yield of fruit probably due to the activity of plant hormones (**Rosaria and Barea, 1975**).

There are other works available on the use of Azotobacterin for different crops. In every case beneficial effects of the biofertilizer have been noted but the effect has been formed to vary from crop to crop, soil to soil and climate.

Chickpea : **Mcurya, B. R. and Sanoria, C. L., 1986**

S. N. F. : **Rim – Yong – dal, 1992**

Tomato : **Yuki *et al.*, 1994**

Tomato : **Terry *et al.*, 1995**

Pea, Onion, cabbage : **Milosevic, N.; Govedorica, M.;**

Bogadanovic, D. and Ubovic, M., 1995

Tomato: **Eclathil, T. T.; Manian, S. and Udaiyam, K., 1996**

Cabbage : **Wange, S. S.; Patil, P. L.; Mehar, B. B. and Karkeli, M. S., 1996**

Rao and Sharma (1981) carried out an experiment with two strains of A.chroococcum on four varieties of wheat. They reported that strain HA-1 had fixed higher amount nitrogen as compared to HA-2 strain. They also reported that the effect of inoculation on tomato increased with decreased application of mineral nitrogen. Application of nitrogen inhibited the proliferation of A.Chroococcum in rhizosphere of tomato.

Dhillon *et al.* (1980) reported that inoculation of maize seed with A.chroococcum gave grain yield of 3.58 t/ha in contrast to 3.39 t/ha, which is without inoculation. Foliar application of Azotobacter may bring good effect in certain crops. **Kannaiyan *et al.* (1980)** reported that 3 foliar applications of 100 ml A.chroococcum culture suspension/m^2 after 15 days of transplantation and some days of interval, increased grain yield by 5.6 percent to 6.7 percent respectively with N P K and P K fertilization and increased straw yield with P K but not with N P K.

Azotobacter inoculation led to an increase in total carotene and β–carotene of carrot (**Khuller and Chahal, 1977**) and often should an increase in protein content of vegetable crops (**Seigal and Schmidt, 1966**); Azotobacter inoculation increased not only the nitrogen content of grain and straw but also the nitrogen assimilation by oat and barley plants (**Klikovs'ka, 1954; 1955**). Azotobacter was effective in increasing significantly the N content of wheat grains when the soil was basal dressed with ammonium sulphate and super phosphates (**Sonoria and Rao, 1973-74**). Fungistatic behaviour of Azotobacter has been observed by some workers. Addition of Azotobacter to soil decreased the number of fungi present. A critical C/N ratio proposed by **Huser (1965)** was 70 – 40 under which Nitrogen fixation in soil stops abruptly. Presence of soil extract in addition to glucose or sucrose or Mannitol in liquid broth, enhances the efficiency and amount of fixed nitrogen (**Sundara Rao and Iswaran, 1959; Abd-EL-Malek *et al.*, 1976**); clay being superior to clay + sand and sand + humified saw dust though beneficial effect of Azotobacter due to its nitrogen fixing ability has often been criticised (**Sinha, 1958; Copper, 1959; Macura, 1966; Misustin, 1970 and Alexander, 1977**) yet the possibility of beneficial effects arising out of this ability can not be ignored. The fixed nitrogen is later made available for use of higher plants (**Sheloumova, 1941**).

In culture medium, Azotobacter can fix 5 to 20 mg N/g of sugar consumed depending upon the species of Azotobacter involved (**Daji, 1970; Sanoria, 1965; Rawat, 1977**). Azotobacter in liquid broth fixed 4.2 mg N/g sucrose consumed (**Bhinde and Purandar, 1979**) and maximum 18 – 60 mg when Mannitol was supplied (**Kasiranjan *et al.*, 1976**). A.chroococcum, isolated from non-saline slightly alkaline soil, was found to have highest efficiency (36.9 mg N/g C) which decreased with increasing salt concentration (**Mohmoud *et al.*, 1978**). Isolates from saline alkali soil of different places did not have much differences in their morphology except in their efficiency; the best two being having capacity to fix 11.6 mg and 9.6 mg N/g C (**Ahmed *et al.*, 1979**).

Kulikovskaya (1954-55) found that Azotobacter treatment of oat and wheat seed increased the nitrogen content of grain and straw, nitrogen assimilation by plant due to Azotobacter treatment being 34.2 to 37.5 percent higher than that by control plants. Root treatment of tomato seedlings with Azotobacter vineludi and Azotobacter beijirinckii accelerated plant growth and increased the fruit yield (**Rosario and Barea, 1975**).

Kholopov (1952) performed field and laboratory experiments on the use of Azotobacterin in Western Siberia. Of the 360 experiments,

he conducted 67 percent experiments on cereal crops and the rest were performed with potatoes, sugarbeat, cabbage, carrot and other plants. In more than 71 percent of all experiments, the use of Azotobacterin brought about yield increases greater than 10 percent. **Roizin and Ezrukh (1958)** obtained similar results with potato, cabbage and oats.

Inoculation of different strains of A.chroococcum was variable on growth of roots and shoots of different types of seeds (**Sanoria and Sundara Rao, 1973-74; Agarwal and Sanoria, 1978**). Peaty Azotobacterın and Azotobacterin alone improved the growth of decorative shrubs (**Manil and Brakel, 1965**) and vine cuttings (**Minasyn and Nalbandyan, 1965**) respectively. Inoculation with strains of A.chroococcum sometimes increased grain yield of maize by upto 5–8 percent; and as the increases never reached 15 – 20 percent as reported by some authors, the suggested possible reason was the prevailing dry conditions. The Azotobacter preparation, produced by the submersion method was not effective in increasing potato yields irrespective of levels of available soil phosphorus and soil pH and was not recommended for the inoculation of potatoes (**Nemec and Recina, 1963**).

In field condition the amount of nitrogen fixed by Azotobacter showed wide variation ranging from 0.1 to 60 kg per hectare annually

(**Greaves and Johnes, 1942; Tzchapek and Garboksy, 1952; Pelczar and Reid, 1958; Paul *et al.*, 1971; Dobereiner *et al.*, 1973**) and from 10 to 35 kg/ha during crop growth in soil and rhizosphere (**Mishustin and Naumova, 1962; Nair *et al.*, 1972**).

Molybdate (**Valodin, 1969**). Soyabean responded better to inoculation (Radicin being more effective than azotogen) than nitrogen fertilization on a laessloam soil (**Streuber, 1967**). **Nair *et al.* (1972)** and **Tchan and Jackson (1966)** observed no significant increases in yield due to Azotobacter inoculation. Yield of potato, tobacco and lettuce were not effected by Azotobacter though it remained in the rhizospheres throughout the growth period (**Manil and Brakel, 1965**).

Several experiments conducted in temperate regions of the world shows that nitrogen fixation in Azotobacter inoculated soil is not more than 10 to 15 kg N/ha/year, depending on the availability of carbon sources. Bacterial preparations containing Azotobacter cell under the name 'azotobacterin' are being produced and used in former USSR and East European countries such as Czechoslovakia, Romania, Poland, Bulgaria and Hungary where bacterization of seed with azotobacterin has proved beneficial in increasing yield of crops such as wheat, barley, maize, sugarbeat, carrot, cabbage and potato. The increase in yield of field crops was not more than 12 percent over corresponding uninoculated controls (**Subba Rao, 1977**).

Field experiments in India on different crops by seed or seedling inoculation with Azotobacter and with or without basal dressing of organic and inorganic fertilizer revealed that yield increases due to inoculation was rather variable for different crops like onion (**Joi and Shinde, 1976**), wheat (**Sundara Rao *et al.*, 1963; Mehrotra and Lahiri, 1971**), Rice (**Mehrotra and Lahiri, 1971**) and sorghum (**Jagdale, 1977**).

The simultaneous inoculation of Azotobacterin and phosphobacterin in takyr (a soil type commonly distributed in the desert part of desert Serozens) soil of the Uzbek S. S. R., increased the number of Azotobacter cell in the cotton rhizosphere; and also promoted the growth of cotton plants (**Kvosnikov, 1958**). In turf-pod soils of the Ukrainian S. S. R. fertilized with organo-mineral fertilizers,, the use of Azotobacterin in a mixture with phosphobacterin yielded somewhat large tomato crop than those obtained with the use of Azotobacterin and phosphobacterin alone (**Linchevskaya and Kaliberda, 1958**).

Kudzin and Yaroshenko (1955) showed that the efficiency of phosphobacterin in steppe-zone soil of Ukrainian S. S. R. could be enhanced with simultaneous use of Azotobacterin. The following increases in potato yield were noted by **Yung (1954)** in turf-podsol soils of the Kirov region, Stalin Collectcive Farm Fslenki Falenki

district; in the presence of phosphobacterin, 21 centres/hectare, in the presence of Azotobacterin + phosphobacterin. 39 centres/hectare; Malodaya Guardiya Collective Farm, Kil Mez district, in the presence of phosphobacterin, 39 centres/hectare, in the presence of Azotobacterin + phosphobacterin, 63 centres/hectare.

Inoculation of Azotobacter into virgin soils of Yenisei Territory fertilized with 60 tonnes manure/hectare increased the potato yield by 15 percent; the increase in the presence of phosphobacterin alone was 6 percent; and in the presence of both it was 30 percent **(Burakova, 1956), Vasakhnil and Manorik (1954)** in USSR increased the effectiveness of compost phosphobacterin. **Yung (1954)** found that simultaneous application of Azotobacter and phosphobacterin was more effective than either fertilizer applied alone. Application of Rhizobium and Bacillus megaterium var. phosphaticum on vigna radiata and Glycine max. significantly higher yield was found due to inoculation of Bacillus megaterium than obtained by Rhizobium. Combined inoculation of Rhizobium and Bacillus with super phosphate and rock phosphate gave significantly high yield of these crops.

There are other works available on the use of phosphobacterin for different crops. In every case, beneficial effects of the biofertilizer have been noted but the effect has been found to vary from crop to

crop, soil to soil and climate, e.g. Onion (**Kumar and Mangal, 1997);** Cabbage **(Verma, T. S.; Thakur, P. C. and Singh, A., 1997).**

Simultaneous inoculation with different microorganisms:

Normally bacterial fertilizers contain a single bacterial species, such as Azotobacterin–Azotobacter, nitrogen–Rhizobium, phosphobacterin–Bacillus megaterium/pseudomonas sp. and silicogen–Bacillus siliceous. In order to stimulate plant growth attempts have been made to use more than one bacterial fertilizer at a time. it has been often observed that Azotobacter is closely associated with the roots of cultivated plants. Some observations illustrating the result obtained from the combined use of Rhizobium and other soil microorganisms are briefly reviewed here.

Allison and Hoover (1934) and **Allison and Minor (1938, 1940)** showed that Azotobacter cultures contained an organic substances required for the growth of leguminous bacteria and named it 'coenzymi R'. According to **West and Wilson (1938)** it was identical to biotin. These stimulating discoveries might have inspired the microbiologists to test the combined effect of Rhizobium and Azotobacter inoculation on crops.

In field experiments carried out in Armenian S. S. R., the use of Azotobacterin alone increased wheat grain yield by 12.5 percent, the

simultaneous use of Azotobacterin and nitrogen increased the yield by 27.5 percent **(Kirakosym *et al.*, 1949)**. Field experiments performed in moderately poetzolized soil of the Kirov region showed that Azotobacterin alone increased yields by 30 percent and use of Rhizobium and Azotobacter together increased the oat yield by 73 percent (**Rubenchik, 1963**).

Shelomova (1941) treated seeds of some leguminous plants with Azotobacterin and root-nodule bacteria specific for plants. He examined increase in yield with simultaneous treatment were greater than those obtained by treating the seeds with root-nodule bacteria alone. In similar trials with oat (**Rubenchik, 1941**), wheat (**Kirakosym *et al.*, 1949**) and lupine and cabbage (**Vigileva, 1954**) yield increases were obtained due to simultaneous inoculation with Rhizobium and Azotobacter.

Vigileva (1954) described symbiosis between Azotobacter and Rhizobium when grown in a mixed culture in Ashby's medium with sucrose. The growth rate of Azotobacter was enhanced. A similar but weaker effect was obtained when dead Rhizobium cells or their metabolic products were added to growing Azotobacter culture. Leguminous bacteria from alfalfa had a greater stimulatory effect than those from lupine. The expenditure of energy sources in mixed culture was smaller than that in pure Azotobacter cultures. In these

conditions, the nitrogen fixing xapacity was enhanced. Since dead Rhizobium cells in Azotobacter cultures enhanced Azotobacter growth, **Vigileva (1954)** put forward a hypothesis on the hormonal activity of Rhizobium. Rhizobium may also effect Azotobacter in different ways. It was shown that Azotobacter grow poorly in the presence of its own culture filtrates but its growth could be enhanced by adding living Rhizobium cells. Vigileva also showed that Azotobacter enhanced the growth of Rhizobium.

The survival of Rhizobium japonicum cultures in peat with Beijerinekia indica and A.chroococcum was examined with special reference to nodulation and yield of soyabean. An increase in yield of the crop was obtained as compared with culture containing R.japonicum alone (**Apte and Iswaran, 1974**). Inoculation of soyabean seeds with combined culture of Rhizobium and A.chroococcum was as good as inoculation with an effective culture of Rhizobium alone in respect of nodulation and yield as referred earlier (**Sharma and Rao, 1978**).

Rubenchick *et al.* (1958) investigated the inter-relationship between Azotobacterin and Bacillus megaterium var. phosphaticum and showed that when the later was present, the number of Azotobacter cells in the rhizosphere of wheat, rye, oat and barley seedlings was higher. Other experiments, however, showed that in the

presence of Azotobacter spore germination of Bacillus megaterium var. phosphaticum was enhanced and the rate of formation of water soluble phosphorus in the soil increased. Moreover, the nitrogen fixing activity of soil was enhanced in the presence of B. megaterium var. phosphaticum the stimulatory effect of B. megaterium var. phosphaticum on growth of Azotobacter and on its nitrogen fixing activity in soil could be due to the fact that the amount of water-soluble phosphorus increased as a result of farmer's metabolic activity, thus creating favourable conditions for Azotobacter's phosphorus nutrition. In addition, enhancement of spore germination and formation of water-soluble phosphorus of B. megaterium var. phosphaticum was apparently with Azotobacter's excretion of biologically active substances.

Pisemskaya and Kochienova (1956) reported that simultaneous inoculation of phosphobacterin and Azotobacter was less effective than inoculation with phosphobacterin and phosphobacterin showed no advantage over their single application (**Boyarovicn, 1954; Gebgardt, 1951; Teklyakova, 1955**). However, the important point in the case of bacterization with Azotobacter (non-symbiotic nitrogen fixing organisms) is that the seeds are to be bacterized with a heavy population. In the USSR, Azotobacter and phosphobacterin were produced for three million ha and 14 million ha

of land, respectively. The results elsewhere gave positive response only in a limited number of cases. At the Indian Agriculture Research Institute (IARI) it has also given positive results in some cases but not in all and its uptake by plants. **Sperber (1957)** suggested that phosphorus was released in soil from inorganic compounds due to local accumulation of lactic acid and action of H_2S produced by microorganisms particularly under anaerobic conditions. **Sen and Paul (1957)** claimed that lactic acid and H_2S developed by microbial metabolism were at least the two biological products important in rendering insoluble phosphate into available form.

The phosphobacterin inoculation caused a decrease in soil pH due to the formation of organic acid (**Sundara Rao and Sinha, 1963**) supports the view that CO_2, H_2S and acids, the end products of microbial activities affect the solubilization of phosphate. However, **Rana *et al.* (1975)** did not find any beneficial effect of phosphobacterin inoculation of wheat on the available phosphorus content of the soil. **Azcon- G de Aguilar (1978)** also did not find any increase in overall pool of soluble phosphorus in the soil. **Kundu and Gaur (1981)** suggested that solubilization process is carried out by diverse physiological groups of microorganisms such as bacteria, fungi and actinomycetes.

Of the three major mineral elements, phosphorus plays both

direct and indirect role in the metabolism of plant and microorganisms, its major physiological role being in certain essential steps is the accumulation and release of energy during cellular metabolism. Researches over the years have clearly shows that phosphorus is essential for various activities of the living cells. Deficiency of phosphorus causes disturbances in one or more vital plant processes including visual symptoms of disorders. The subject has been ably reviewed by **Stewart and Williams (1942), Dean and Freid (1952)** and **Mitchel (1953)**.

Insoluble inorganic compounds of phosphorus are largely unavailable to plant and the transformation of available form of applied phosphorus to unavailable form is a major problem but fortunately many microorganisms can bring the unavailable phosphate into solution state (**Goswamy and Sen, 1962; Subba Rao and Bajpai, 1965; Poul, 1966; Taha *et al.*, 1969; Bordiya, 1970** and **Gaur *et al.*, 1973**). The phosphate dissolving bacterial play an important role in supplying the growing plants with their needs of phosphorus and therefore, may be considered to function as plant nutrient factory right at the form.

In the Soviet Union and several eastern European countries, soil were inoculated with bacteria which apparently increased the availability of native as well as applied phosphorus to the plants

(**Smith *et al.*, 1961**). The increase in soil phosphorus resulted primarily from the decomposition of organic phosphorus compounds (**Mishustin and Naumova, 1962**) and was most effective in neutral and somewhat alkali soils.

Effect of microphos inoculant (phospho-bacteria) on grain yield of crop in multilocational trials

Crop	Number of trials	Percent increase due to inoculant	
		P_0	RP_{60}
Paddy	7	10 – 20	5 – 15
Wheat	8	7 – 42	5 - 50
Chickpea	3	10 – 30	15 - 25
Lentil	2	-	13 - 15
Potato	2	30 – 50	-

Phosphobacterin Inoculation:

Insoluble inorganic compounds of phosphorus are largely unavailable to plant and the transformation of available form of applied phosphorus to unavailable forms is a major problem in intensive agriculture, in relation to phosphate fertilization programme, but fortunately many microorganisms can bring the unavailable phosphate into solution (**Goswamy and Sen, 1962; Sundara Rao and Sinha, 1963; Subba Rao and Bajpai, 1965; Paul, 1966; Taha *et al.* 1969; Bordiya, 1970; Gaur *et al.*, 1973**). The phosphate dissolving

bacteria play an important role in supplying the growing plants with their needs of phosphorus and therefore, may be considered to function as plant nutrient factory right at the form.

In the Soviet Union and several eastern European countries, soil were inoculated with bacteria which apparently increased the availability of the native as well as applied phosphorus (**Smith *et al.*, 1961**) to the plants. The increase in soil phosphorus compounds (**Mishustin and Naumova, 1962**) and was most effective in neutral and somewhat alkali soils.

Effectiveness of phosphobacterin on growth and yield of crops has been reported from Russia and its use is also popular in Russian agriculture. Most of the workers in other parts of the world however, reported negative response, which has been attributed largely due to the use of unsuitable cultures. The use of phosphobacterin culture in legumes seems to have been started in the recent past and as such the information in this respect seems to be meagre.

This is a controversy whether phosphobacterin treated seeds results in poor growth in the early stage or not. **Gopal Krishnamurthy *et al.* (1967)** reported that the bacteria of phosphobacterin got established in the rhizosphere and continue to multiply upto 70 days and their number was reduced thereafter. Meanwhile they needed additional phosphorus for their body

development and completed with plant for phosphorus. In the early stage of plant growth, they observed poor vigour of rice plants treated with phosphobacterin culture. **Mishustin and Naumova (1956)** observed better growth of cabbage (*Brassica oleracea* var. Capitata) and tomato (*Lycopersicum esculentum* Mill.) plant when inoculated with phosphobacterin.

In a green house trial with pea (*Pisum sativum*) **Yousry *et al.* (1978)** reported that phosphobacterin inoculation increased plant dry matter yield by 10.9 percent when compared with uninoculated control. **Pikovskaya (1948)** obtained an increase in green matter yield in oat plants due to the inoculation of phosphobacterin increased lime and pine weights at their intensive growth period by 22 – 25 percent and 16 – 19 percent, respectively.

Soyabean yield increased from 17.40 q/ha to 19.89 q/ha when the seeds were treated with phosphobacterin (**Marinova, 1970**). Phosphobacterin inoculation increased the growth and yield of soyabean (**Arora, 1975**) broad bean (**Taha *et al.*, 1969**) maize (**Sharma and Singh, 1971**) and chick pea (**Ahmad and Jha, 1977**).

Sundara Rao (1965) reviewed the results of field trials conducted with phosphobacterin inoculation in wheat, berseem, maize, black gram and rice in different parts of the country. He reported that in 10 out of 18 experiments, phosphobacterin

inoculation showed beneficial effects on the yield. On the other hand, **Bordiya (1970), Arora (1975), Rana *et al.* (1975)** and **Swaby and Sperber (1958)** did not find significant effect of phosphobacterin on grain and straw yields of wheat and green gram.

Yield increases from the use of phosphobacterin ranged from 0 to 70 percent (**Smith *et al.*, 1961**). However, the average increase was about 10 percent. It was also found that yield of tomato grown in green house with phosphobacterin inoculation increased upto 7.5 percent (**Smith and Allison, 1962**). Observing the ineffectiveness of phosphobacterin in very dry autumn or in systematically fertilized soil, **Kudzin and Yaroshevich (1962)** reported that phosphobacterin inoculation increased yield of winter wheat and maize by 2.2 and 2.3 q/ha, respectively.

Shah (1959) reported 20 percent increase in berseem fodder yield due to phosphobacterin inoculation. Marked increase in the dry mater accumulation and yield of cowpea was observed by **Bajpai (1965)** and **Bajpai and Sundara Rao (1971)**.

Gerresten (1948) reported that CO_2 produced by microorganisms in rhizosphere seems to be an effective mechanism in increasing the availability of phosphorus.

Azotobacter is an aerobic heterotrophic organism and therefore,

oxygen is required for ATP formation, but nitrogen fixation is an anaerobic process. Obviously, must be excluded from the site of nitrogen fixation. Various proposals have been offered from time to time to explain the oxygen protection mechanism, the presence of membranes around nitrogenase to prevent oxygen damage and conformational protection of nitrogenase by rearrangement of the physical structure of nitrogenase so as to afford moderate resistance to oxygen, and the presence of large amount of slime around bacterial cell which minimize oxygen entry (**Subba Rao, 1994**).

The lack of organic matter in soil is a limiting factor in the proliferation of Azotobacter in soil. The beneficial effects of small amounts of humus on the growth of Azotobacter and its nitrogen fixation are known.

Effect of sodium humate and fulvic acid on the growth of Azotobacter chroococcum and its nitrogen fixation:

Sodium humate or fulvic acid added (ppm)	Sodium humate 2		Fulvic acid	
	Growth (Cell number x 10^6 / ml)	N Fixed (mg/g sugar oxidized)	Growth (Cell number x 10^6 / ml)	N Fixed (mg/g sugar oxidized)
0	2.4	11.5	2.4	12.0
20	4.3	12.0	6.1	14.0
100	8.3	13.0	12.0	15.0
200	20.6	15.5	25.0	16.0
300	36.0	16.0	37.0	17.0
500	40.0	18.6	38.0	19.6
700	35.0	18.3	38.0	19.9
1000	25.0	15.9	28.0	17.5

Azotobacter Inoculation and its effect on Crops:

It is well known that symbiotic bacteria improve the soil properties and quality and quantity of vegetables but asymbiotic organisms particularly Azotobacter have also shown their utility. Almost from the time that Azotobacter was first discovered by **Beijerinck (1901)** as a micro-organism capable of fixing free Nitrogen. Statements have been made that this bacterium is closely associated with cultivated plants and inoculation of soil (**Gerlach and Vogel, 1902**) and plants (**Voorhees and Lipman, 1907**) may benefit both soil and plant productivity. Results of **Kostychiv *et al.* (1920)** and **Truffaunt and Bezssonoff (1924)** further promoted the research with Azotobacter. Active work on non-symbiotic bacterial inoculant was initiated from 1932 onwards by a number of Soviet Scientists and in 1942, 'Azotogen' a commercial preparation of A.chroococcum was used on five million acres of crops (**Balls, 1946**).

A number of experiments were also conducted in some other countries. Results of most of the later experiments were reviewed by **Cooper (1959)** and **Mishustin and Naumova (1962)**. A monograph 'Azotobacter and its use in Agriculture' by **Rubenchik (1960)**, later translated from Russian into English in 1963, incorporated much information on the structure, metabolism and ecological relationships along with its use as bacterial fertilizer.

The main use of Azotobacter and Rhizobium in Agriculture is in the form of 'azotobacterin' and 'nitragin' respectively. Statements have been Rhizobacterin often made that Azotobacter inoculation of soil or seed is effective in increasing yield of crops in well-manured soil with high organic matter content. Besides the ability to fix atmospheric nitrogen, this micro-organism is also known to synthesize biologically active substances such as vitamin-B complex, indole acetic acid and gibberellins and has fungistatic properties on certain pathogenic fungi. Due to these attributes Azotobacter showed beneficial effects on seed germination, plant growth, plant stands and vegetative growth (**Subba Rao, 1977**).

Thompson (1974) obtained significantly increased yield from wheat and barley, and the yield components were highly influenced by Azotobacter inoculation. Yield of paddy was increased more by application of Azotobacter than with the application of 50 kg N/ha (**Patil *et al.*, 1976**). Seed, seedling and soil inoculations with Azotobacter were reported to increase the growth and yield of many crops, viz. paddy, wheat, maize, sorghum, cotton, tobacco, sunflower and mustard (**Brown *et al.* 1962 and 1964; Sundara Rao *et al.*, 1963; Rovira, 1963; Mehrotra and Lehri, 1971; Patel, 1969; Mishustin, 1970; Jain *et al.*, 1973; Muthukrishnan *et al.* 1975; Oblisam *et al.*, 1976; Rangarajan and Muthukrishnan, 1976**).

Inoculation with A.chroococcum resulted in increased paddy yield in presence of phosphate fertilizers or lime (**Sulaiman, 1971**) and significant increase of wheat yield was obtained when soil was basal dressed with $(NH_4)_2SO_4$ and yield components of wheat and barley were highly and positively influenced by Azotobacter inoculation **(Thompson, 1974)**, yield of potato was more due to seed bacterization with Azotobacter and ammonium.

Surendra *et al.* (1993) found that the combined inoculation of Rhizobium and phosphobacterin at 50 percent level produced pod yields at par with uninoculated control at 100 percent P application indicating a 50 percent saving of phosphate fertilizer.

Sperber *et al.* (1858) reported that the increase in crop growth due to combined inoculation of biofertilizers might be due to growth regulators produced by Rhizobium and bacillus and also might be due to the solubilisation of insoluble phosphates by production of various organic acid.

The microbial solubilization of inorganic phosphorus fixed in soil to a more labile and available form of phosphorus is well established (**Gaur and Gaind, 1993; Sardina *et al.*, 1986; Singh a, 1984; Reddy and Reddy, 1995**).

Bhandal *et al.* (1989) found that single inoculation either with

Rhizobium or PSB and dual inoculation with both of them had significant effect on plant height and dry matter accumulation at pod-filling stages and at flowering stages in pea. Single inoculation were distinctly inferior to the combined inoculation. This could be ascribed to enhanced availability of N in Rhizobium inoculation, P in PSB inoculation and both N and P in dual inoculation plots.

Alagawadi and Gaur (1988) reported that single and dual inoculation with Rhizobium and PSB improved the yield attributes and yield of pea. Associative effect of Rhizobium and the PSB in dual inoculation resulted in significant increase in yield attributes compared to their single inoculations. This could be owing to increased and balanced availability of both N and P in dual-inoculation plots. More pronounced effect of combined inoculation over single inoculation was also observed in gram or chickpea.

Elenkala *et al.* (1985) reported that increased N and P uptake by the crop was observed due to dual and single inoculations with Rhizobium and PSB. Dual-inoculation with Rhizobium and PSB had relatively greater increase in N and P uptake than their individual inoculations. Similar behaviour of three inoculations on nutrient uptake was observed.

Haque *et al.* (1988) reported that studies on test weight of seed of soyabean as influenced by biofertilizers also expressed a significant

effect of biofertilizer of soyabean treatments.

Srivastava and Ahlawat (1995) found higher solubilization of N, P and K from soil by the biofertilizers.

Kumrawat (1997) reported that seed yield and test weight of pea increases significantly due to application of Rhizobium, Phosphate solubilizing microorganisms and N P K fertilizers.

Sharma and Namdeo (1999) reported that the combined application of Rhizobium + FYM + PSB gave the highest uptake of NPK nutrients. Biofertilizers with or without FYM increased N and P status but showed fluctuating trend.

Mikanova *et al.* (1995) studied that yield of pea increased with the use of P-solubilizing inoculants in the absence of fertilizers to a level similar to that obtained with 41 kg/ha alone.

Tomer (1998) demonstrated that application of PSB (10 g/kg seed) in black gram gave the maximum grain and straw yield when used in combination of FYM (5 t/ha). However, PSB singly also gave a significant increase in yield over control.

Srivastava *et al.* (1998) reported PSB increases nodulation, nitrogenase activity, growth and grain yield of pea.

Jain, Joshi and Taneja (1988) in experiments on effect of phosphorus and Rhizobium cultures on protein and gum content of

clustger-bean (*Cyamopsis tetragonoloba* L. Taub) have reported the application of 20 kg P_2O_5/ha to C. tetra gonoloba increased both the gum and protein content in seed inoculation or 40 – 60 kg P_2O_5 increased the protein content but decreased the gum contents.

Bandopadhyay (1988) in experiment with variation in host and bacteria in the nodulation of phaseolus species reported seeds of 8 varieties of P- vulgaris were sown and inoculated separately with 13 strains of Rhizobium paheoli. Seeds of two varieties of P-aureus (Vigna radiata). 2 if O-mungo (V-Mungo) and one of P-frilobus (V.aconitifolia) ever inoculated separately with 10 different strains of cowpea Rhizobium. At flowering, nodules were harvested and fresh weight measured. From the analysis of variance of number of nodules and nodule fresh weight it was found that both host variety and bacterial strain produced significant effect on the above 2 characters.

Gupta and Sharma (1989) in experiment with interactive effect of Rhizobium and Phosphorus on nodulation, crop yield and nitrogen fixation in lentil (*Lens culinaris* M) have reported in field trials in the Rabi (winter) season of 1985-86 on sandy loam soil. Nodulation and N-fixation of lentils given 0, 8, 16, 24 or 32 kg P/ha and seed inoculation with Rhizobium or no inoculation were studied 30, 60 or 90 days after sowing. The highest nodule number and nodule DM/plant were found at 6 DAS with inoculation and P application,

seed yield was 0.87 – 1.30 t/ha with 0.32 kg P and no inoculation and 0.89 – 1.68t with 0 – 32 kg P and inoculation. Seed protein content increased with P application and inoculation. N fixation ranged from 0.41 N/plant per day at 30 DAS with no P or inoculation to 1.25 mg/plant at 60 DAS with 32 kg P and inoculation.

Singh and Singh (1989) in experiment on effect of nitrogen, phosphorus and seeding rates on growth, yield and quality of guar and rainfed conditions have observed in 1981 – 82, *Cyampsis tetragonoloba* gave average seed yields of 1.89, 1.54 and 1.90 t/ha with seed inoculation with Rhizobium Japonicum. 20 kg N/ha and inoculation + N respectively; compared with 1.42 t in the untreated control yields were 1.39, 1.69 and 1.92 t with 0, 30 and 60 kg P_2O_5/ha respectively and 1.41, 1.68 and 1.91 t with sowing rates of 10, 20 and 30 kg/ha, respectively. Effects of inoculation and/or N and P treatments on nodulation, root dry weight, seed weight/plant, seed protein and gum contents, 1000 seed weight, water use efficiency and net returns were similar to those on yields.

Singh and Singh (1990) in experiments on uptake of nitrogen, phosphorus and potassium by guar (*Cyamopsis tetragonoloba* L. Talib) as influenced by nitrogen (with or without seed inoculation) phos and seeding rates under rainfed conditions reported that in 2 years trials (1981 – 82) with *C. tetragonoloba*, seed inoculation with and uptake in

seeds and straw, followed by seed inoculation and N. Increasing P_2O_5 rates (0, 30 and 60 kg/ha) increased both contents and uptake of NPK. Effects of sowing rates (10, 20 and 30 kg seeds/ha) on nutrient contents and uptake were inconsistent in the two years.

Sidorova, Simakov and Stolyarov (1990) in experiment on evaluation of pea varieties for nitrogen fixing activity reported that there were marked inter-varietal differences in N fixation activity between 13 high-yielding varieties in pot tests using seed inoculated with Rhizobium leguminosarum strain 250, N fixation per plant was 0.09 – 18.35 g N/ha when mineral N was applied and 43.49 123.45 g N/ha when it was not use of N fertilizers caused a considerable fall in N fixing activity, especially in Neosypayuschisya and Touzhenik, which appreciably exceeded other varieties in N fixing activity when no N fertilizer was applied. An increase in growth period duration, number of fertile nodes per stem/seed yield and plant height was associated with increased N fixing activity and these traits could be used as criteria for selecting lines for high N fixing activity.

Bell, Edwards and Asher (1990) in experiment on growth and nodulation of tropical food legume in dilutes solution culture reported that twenty-two tropical food legumes were grown in dilute nutrient solution with or without Rhizobium inoculation (Bradyrhizobium japonicum in soyabeans, Rhizobium leguminosaram in chick peas, R-

legumiso sarum biovar phaseoli in phaseolus vulgaris cv. Gallary and Bradyrhizobium spp. in all other species) and suplied with either low adequate amounts of inorganic N. Growth of legumes supplied with adequate inorganic N was generally satisfactory. However, the solution P concentration (15/M) was excessive for vigna mungo, while the initial solution magnesium concentration (1.8/M) was excessive for V. radiata. Growth response to inoculation with Rhizobium at low inorganic N supply were obtained in only 9 of the 22 legumes studied and shoot dry matter yields were S 51 percent of those obtained with adequate N supply. Poor growth by inoculated plants with a low N supply was attributed to failure of the inoculated strain of Bradyrhizobium to infect roots in phaseous lunatus and Pachyrhizus erosus, to low nodule numbers in V. radiata, V. mungo and P. Vulgaris cv. Gadl Groy, or to excessive P uptake in V. Vita 4 and/or Mn uptake in V. mungo and V. radiata. High solution temperature could have limited N fixation by some of the legumes particularly chick peas.

Saber and Kabesh (1990) have observed in a green house pot experiment lentils cv, Giza 9 seed inoculated with Rhizobium legumino sarum were given 15, 100, 200 or 400 kg rock phosphate/fedden with or without a 1.1 mixture of elemental S and rock phosphate and with or without a 1.1 mixture phosphate

dissolving bacterial plant dry weight and N, P, Fe, Zn, Mn and Cu uptake increased with rock phosphate, sand, phosphate-dissolving bacteria compared with the untreated control Dry matter yield and nutrient uptake were slightly higher with S application than phosphate-dissolving bacteria.

Parthiban and Thamburaj (1991) in experiment on influence of Rhizobium culture and nitrogen fertilization on French beans have observed in trials with the *Phaseolus vulgaris* cv. Premier, under partial irrigation and plants received N at 0, 25, 50, 75 or 100 kg/ha + P at 100 kg/ha and K at 50 kg/ha Rhizobium sp. culture inoculation. The number of days to 50 percent flowering was increased by high N rates, from 38 days in controls without any fertilizer or inoculum to 48 days at the highest N rates. The highest yield of green (21.46 t/ha) was obtained with 50 kg N/ha Rhizobium inoculation.

Chandra (1991) in experiment on influence of different levels of Rhizobium inoculum and phosphorus on nodulation, dry matter production and yield of lentil observed in field trials in 1987 – 90 at Nayina, Uttar Pradesh, lentils v. PL 406 gave seed yields of 1.05, 1.16, 1.29 and 1.24 t/ha with no inoculation and seed inoculation with 5, 50 or 100 g Rhizobium inoculum/kg seed, respectively and 1.03, 1.19 and 1.33/ha with application of 0, 20, and 40 kg P_2O_5/ha respectively inoculation increased nodule number and DW in 2 out of 3 years and

P application increased nodule number only in 2 out of 3 years.

Azad (1991) in response of phosphorus and Rhizobium culture on grain yield of lentil observed in field trials at 3 locations in Gurdashpur district in Punjab in 1986 – 87 Lens culinaris CV L 9-12 was inoculated or not inoculated with Rhizobium and given 0, 20, 40 or 60 kg p/ha. Grain yield was increased by P irrespective of Rhizobium inoculation. Rhizobium inoculation increased mean yield over uninoculated controls at all levels of P application. Highest yield (1.56 t/ha) was with 60 kg P/ha and Rhizobium inoculation. Yield ranged from 0.8 t without P to 1.39 with 60 kg P without Rhizobium inoculation and from 1.06 to 1.56 t with Rhizobium.

Roy and Rohaman (1992) on effect of seed rate and inoculation on nodulation, growth and yield of lentil have observed fully replicated randomized field trials were run during the winter seasons of 1989 – 90 and 1990 – 91 at Joydebupur Bangladesh on clay loam soil. Two lentil cultivars were grown (lintil-5 and L-81124) under 3 sowing rates (10, 20 and 30 kg seed/ha) and 3 inoculation treatment (now, inoculation with Rhizobium strain RLC-140 and inoculation + 20 kg N/ha at sowing). For both species LAI and DM increased with increasing sowing rate but the number of nodules/plant fill. Lentil-5 produced more DM. Seed yield increased linearly with increasing sowing rate with inoculation and with N application. Apparent harvest

index was influenced only by sowing rate.

Yanni (1992) in performance of chick pea; Lintil and Lupin nodulated with indigenous or inoculated Rhizobia micropartners under nitrogen boron, cobalt and molybdenum fertilization schedules observed chick peas, lentils and lupinus albus grown in pots were soil inoculated with Rhizobium loti, R. legumino surum biovar areas and Bradyrhizobium sp., respectively with or without 30 or 60 ppm. N. Plant were also given 3 ppm. Mo, 2 ppm CO_2 + or 1 ppm. -B or no trace elements. Nodulation plant DW and seed yield where increased by inoculation. Nodulation in chick peas and L. albus was decreased. But seed yield increased by 60 ppm. compared with 30 ppm. N. The effects of trace elements on inoculation and seed yield varied with species, N rate and inoculation treatment results are tabulated. Seed yield was generally highest in plant given Mo.

Csizinszlay (1992) on growth and nutrient accumulation of winged been, Psophocarpus tetragonolobus (L.) DC. Seedlings with seed inoculation and various nitrogen sources observed P. tetragonolobus strain Tpt-1 seedling from non-treated seeds or seeds inoculated with the cow pea strain of Rhizobium sp. were grown in a greenhouse, with 4 N treatments (100 percent NO_3, 70 percent NO_3, 30 percent NH_4, 50 percent NO_3, 50 percent NH_4 and 100 percent Urea). Plant height were measured weekly and numbers of tritaliate

leaves were counted from 3 weeks after sowing. Shoots were cut off 6 weeks after sowing and BW. was recorded. Shoots were than dried at 60 percent C and their DW contents of N, P and other elements were determined. Root DW and element content were also determined. After 45 percent days roots nodules were most numerous with inoculum and 50 percent NO_3, 50 percent NH_4–N, and Zn concentrations in shoots and Mo concentration in root were higher with then without inoculum. Plants were tallest and number of trifoliate leaves greatest with 70 percent NO_3; 30 percent NH_4 – N treatment plant height number of trifoliate leaves. Urea reduced plant height, Number of trifoliate leaves and shoots and root DW. seedlings from non-inoculated seeds required a 70 : 30 ratio of NO_3; NH_4 – N for optimum growth and development.

Kumar and Agarwal (1993) in experiment on response of lentil to Rhizobium inoculation, Nitrogen and Phosphorus fertilization observed in a field trial in the winter season of 1989 – 90 on loam soil at Gurukel-Narsan. Uttar Pradesh lentils CU. T.36 were inoculated with Rhizobium or not inoculated and were given 0 or 20 kg N/ha and 35 – 100 kg P_2O_5/ha. Seed yield was increased from significantly different between inoculation treatments (2.03 – 2.29 t/ha in year 1 and 1.77 – 1.87 t in year 2) or between P treatment in year 2 but ranged significantly from 2.15 t with no P to 2.25 to with 40 kg P_2O_5

in year 1 and there was a significant interaction between inoculation and P rate in year 1 only.

Katyal, Venkateswarly and Das (1994) in experiment with biofertilizer for nutrient supplementation in dryland agriculture. Protentials and problem have observed the potential and limitation of Biofertilizer (BF) reviewed.

Past research has shown a general inconsistent response pattern to biofertilizer treatment of dryland crops. Key factors contributing to the inconsistent performance of BF in drylands are highlighted. Some specific suggestions on future areas of research to optimize the response to BF in dryland agriculture are presented.

Fernandez and Felips (1994) in experiment on essentiality of boron for symbiotic denitrogen fixation in pea (Pisum Sativum) Rhizobium nodules have observed the effect of B deficiency on symbiotic Nitrogen fixation in pea cv, Argona was studies. In the absence of B the number size and weight of nodules decreased and nodules development changed leading to an inhibition of nitrogenous activity. Examination of B-deficient nodules showed dramatic change in cell walls and in both peribacteroid and intertion thread membranes. Suggesting a rate for B in the stability of these structures. It is concluded that B is required for normal development and function of nodules.

Hegle and Dwivedi (1994) in experiment with crop response to biofertilizer in irrigated areas have observed biofertilizers have an importance rate to play in improving nutrient supplies and their crop availability. They are of Particular significant in intensively cultivated irrigated areas where in a wide demand-supply gap of plant nutrients exists for very high nutrient turnover in the soil plant system. Experiment conducted under All India coordinated Agronomy Research Project and other programmes in different crops and agroecologies have proved the potentiality of biofertilizer as a important ingredient of integrated plant nutrient supply systems. However, the experimental results clearly indicate that crop responses to biofertilizer are highly inconsistent and unpredictable, thus emphasising the need for refinement in biofertilizer production, distribution and use techniques at both research and development fronts. The present article is confined to a critical review of the performance of different biofertilizer under actual field conditions in irrigated areas. Some important areas for future research to improve the agronomic efficiency of biofertilizer are also indicated.

Laura, Raj and Sangwan (1994) in experiment on effect of inorganic, organic and biofertilizer on pearl millet yields in dryland areas have observed Pearl millet (Pemisstum glaucum) cv. HHB-67 was grown in plots on a sandy loam soil from 1988 to 1991 to

evaluate the effect of 0, 10, 20 or 40 kg N/ha, 0, 2, 4t FYM/ha and biofertilizer (control, seed inoculation with Azotobacter, seed inoculated with Azospirillum) on yield. The grain yields increased with all N rates upto 40 kg N/ha. FYM increased yields in 3 of 4 study years. Seed inoculation only increased yields by upto 0.22 t/ha as soil temperatures were lower than optimum for the microbes used.

CHAPTER – III

MATERIALS

AND

METHODS

MATERIALS AND METHODS

FIELD EXPERIMENT

i) Experimental Sites:

The sites for field trial was experimental farm of the Sheila Dhar Institute of Soil Science, which is located near Mumfordganj at Allahabad. It is irrigated by tubewell water supplied by the Jal Sansthan, Allahabad.

The first and second field experiments were conducted in the year 2000 – 2001 and 2001 – 2002, respectively.

ii) Field and their Cropping History:

All the experiments were conducted in well levelled square fields with necessary facilities of irrigation. The cropping history of the fields for the preceding two years is given in the following table:

Table – A Field experiments conducted in experimental plots during 2000 – 2002

Year	Kharif crops	Rabi crops
2000–2001	Fallow	1. Wheat (Test crop) *Triticum aestivum* 2. Pea (Test crop) *Pisum sativum* L.
2001–2002	Fallow	1. Wheat (Test crop) *Triticum aestivum* 2. Pea (Test crop) *Pisum sativum* L.

iii) Climatic condition:

The climatic condition of Allahabad is known for its cold winters and almost intolerable summers. However, the rainy season is pleasant. The average rainfall is about 80 – 100 cm and average temperature varied from 32.4 to 40.0°C with mean humidity of about 64 percent.

iv) Soil:

The soil of Sheila Dhar Institute experimental farm is Sandy Clay Loam (alluvial soil) having pH 7.5.

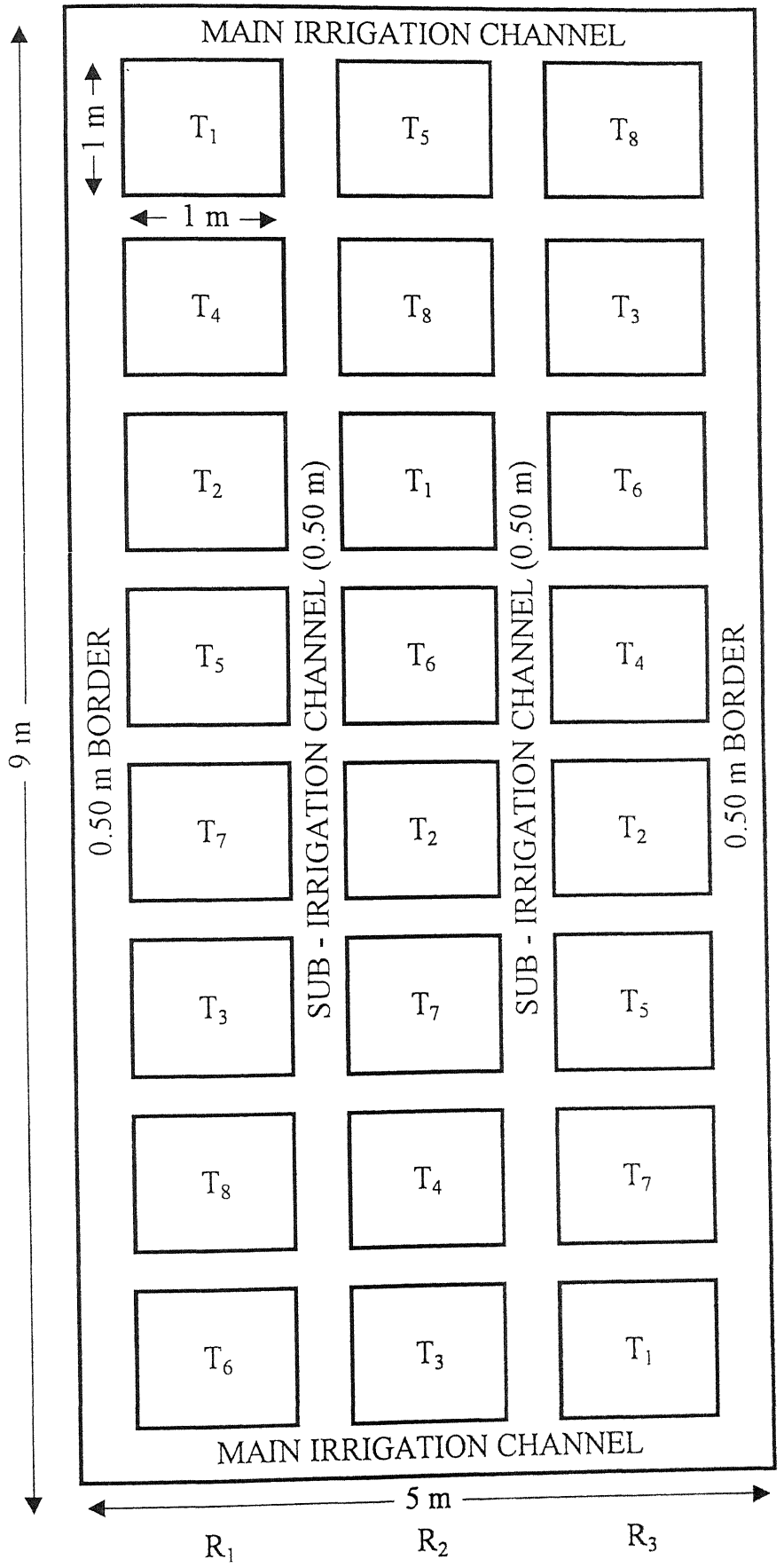
v) Layout:

Randomised Block Design was followed in all the experiments with 8 treatments having 3 replications in plot of 1 x 1 m in the first, second (2000–2001) and first, second (2001–2002) field experiments.

In general, Rhizobium combination with Mussoorie Rock Phosphate (M R P) and Azospirillum & phosphate soluble micro-organisms in the Pea (Pisum sativum L.) field experiments for the year 2000 – 2001 and 2001 – 2002.

The second experiment conducted with Azotobacter combination with Azospirillum, M R P and P S M in the wheat (*Triticum aestivum*) field experiments for the year 2000 –2001 and 2001 – 2002.

Fig. 3.1 Layout Plan of Experimental Field



vi) Basal Dressing:

Before sowing, each plot was basal dressed with additional dose at the rate of 30 kg N as Urea/ha, 60 kg P_2O_5 as M R P/ha and 60 kg K as MoP/ha was simultaneously applied according to the treatments. The required quantities of the fertilizers were mixed well for each plot and mixture was placed in the opened furrows as uniformly as possible by hands. Then it was lightly covered with loose soil in order to avoid direct contact of the inoculated seeds with the fertilizers.

vii) Seed Sowing:

Certified seeds of Pea (Pisum sativum L.) variety Azad P_1 of National Seed Corporation was used. The seed was treated with different biofertilizers as per treatments details and sown in each plot covered with soil. The sowing was done on 1st December 2000 @ 100 kg/ha and second experiment on wheat (Triticum aestivum) variety 'Sonalika' was sown 125 kg/ha in furrows on 4th December 2000.

viii) Procedure of seed inoculation:

For seed bacterization required and measured quantity of seeds were taken in a plastic bucket (25 litre capacity). Recommended quantity of individual biofertilizers (500 g/kg seed) was added in the bucket, thoroughly shaken and properly mixed with the seeds of each variety. After little dryness, proper care was taken for uniform

distribution of bacterial cells on seeds. After a little drying in shade the seeds were sown immediately in the marked plots.

POST-SOWING OPERATIONS

Irrigation:

Irrigation was avoided till the germination completed and plant attained 2 – 3 leaf stage. However, a light flood irrigation just to soak the top soil was given for complete germination after 15 days of sowing. After complete germination, four irrigations were carried out at 25 days of interval.

Inter-culture:

After 8 – 10 days of the irrigation, the soil became compact. Therefore, the hard crust was loosened by Khurpi. Besides aeration, this operation also served the purpose of weeding. At a later stage, 1 or 2 hand weedings were done.

Harvesting:

The crop was harvested at green pod stage for vegetable purpose. The picking was done when pods started changing their colour from dark to light green and pods were completely and tightly filled with grains and pod surface was still smooth. Three pickings were done at an interval of 7 to 10 days. The average yields of green pods were recorded for statistical analysis.

Biometric Observations:

Due to large population of plants in plots, it was rather difficult to record the observations on all plants. Hence, techniques for random sampling was adopted for recording the observations on various morphological, physiological and biochemical characters of pea plants during the course of investigations. The growth indices of plant were recorded at regular intervals throughout their life cycle to measure the relationship between growth attributes and final yield. Such measurements were made at successive intervals of plant growth after sowing. A sample of five plants from each plot was drawn at random to record the morphological characters.

(i) Height of the Plants:

The observations on height of the plant was recorded during active growth stage (30 days after sowing) and at pod filling stage (60 days after sowing). The height of the plants were measured in on from the portion of the stem above the soil upto the terminal pair of leaflets on the main shoot excluding tendrils.

POD STUDIES:

(i) Number of pods per plant:

For the study of number of pods, five randomly selected plants from each plot were chosen and pods from each plant were counted and recorded.

(ii) Length of pods:

The pods were picked at random from each plot, their length was measured in cm and the mean values were worked out.

(iii) Number of grains per pod:

The average number of grains per pod was worked out by counting the number of grains in five selected plots. The pods were split open and then number of grains was counted and expressed as number of grains per pod.

(iv) Yield:

The green pods were harvested when they were ready and weight was recorded for each picking to determine the total yield per plot in kg and were further converted to yield in q/ha.

2. Number of nodules:

For studying the nodules, samples were taken at pre-flowering stage. Five plants were randomly uprooted from each plot with the help of Khurpi by digging the soil upto a depth of 15 cm with surrounding area of 10 cm radius from the plant. Roots were washed in running water to make them free from adhesive material of soil particles. During sampling, all possible precautions were taken to minimise the breakage of roots and loss of nodules. In order to avoid shrinkage of nodules, the roots along with nodules were collected in

an ice-box and carried from field to laboratory. The nodules were carefully detached one by one from the roots with the help of a forceps and the number of nodules was recorded. In laboratory, nodules were dried by soaking in filter papers and were weighed with the help of a chemical balance. The data obtained from each plant of each plot were averaged.

Fresh and dry weights:

For fresh and dry weight of plants, five plants were selected randomly from each plot. For dry weight, the plants were dried in sun for two days followed by oven drying at 55°C to 60°C for 3 days and then observations were recorded.

Agronomic Management:

Few days before sowing, the fields were irrigated to maintain optimum moisture in all plots. The time-schedule for the management operations including sowing and harvesting was as follows:

Operation		**Pea**	**Wheat**
Sowing		1st December 2000	4th December 2000
Irrigation	I	16th December 2000	24th December 2000
	II	11th January 2001	20th January 2001
	III	5th February 2001	15th February 2001
	IV	2nd March 2001	5th March 2001
	V	-	30th March 2001
Weeding	I	5th – 10th January 2001	20th – 22nd March 2001
	II	12th – 17th March 2001	5th – 7th February 2001
Harvesting		28th March 2001	10th April 2001

Preparation of soil samples for Analysis:

The representative soil samples of about 1.0 kg from each plot was brought to the laboratory and air dried in shade. Wooden hammer was used for crushing the clods. After thorough mixing they were ground and then passed through 2 mm sieve. The unsieved particles were again crushed thoroughly, mixed and finally passed through the same sieve. The soil sample thus prepared was kept in the same polythene bags and stacked in soil racks for analysis.

METHODS EMPLOYED FOR SOIL AND PLANT ANALYSIS

SOIL ANALYSIS

i. Mechanical Analysis:

Mechanical analysis was done by international pipette method as outlined by **Piper (1963)** for the determination of sand, silt and clay.

ii. pH (1 : 2.5 Soil – water suspension):

pH value was measured with the help of Systonic Digital pH Meter 335

iii. Electrical Conductivity:

Electrical Conductivity (dSm^{-1} at 25°C) of saturation extract was determined with the help of conductivity bridge as outlined by **Jackson (1973)**.

iv. Organic Carbon and Organic Matter:

The organic carbon and organic matter was determined by the modified Walkley and Black rapid titration method (**Piper, 1963**), in which a known amount of the soil was digested with potassium dichromate and sulphuric acid. The excess of chromic acid was back titrated with standard ferrous ammonium sulphate.

v. Cation Exchange Capacity:

Cation exchange capacity was determined by the method described by **Jackson (1973)**.

vi. Total and Available Nitrogen:

Total Nitrogen was estimated by micro Kjeldahl method and available Nitrogen was estimated by the method described by **Jackson (1973)**.

vii. Available Phosphorus:

Available phosphorus was estimated by Olsen's method using Spectrophotometer (**Kanwar and Chopra, 1976**).

viii. Potassium:

Potassium of soil was estimated by the method described by **Jackson (1973)**.

PLANT ANALYSIS

5 g of oven dried plant material was taken in 100 ml of tri-acid mixture made from 750 ml of conc. HNO_3, 150 ml of conc. H_2SO_4 and 350 ml of 60 percent per chloric acid was adopted. The content was heated on a hot plate at low heat for several minutes and then the temperature was increased. Heating was done till the H_2SO_4 evolved. The volume was then reduced to about 3 to 5 ml but not allowed to dry. Then distilled water was added to the beaker and contents filtered through an acid washed filter paper into a volumetric flask and volume was made up with distilled water.

ANALYSIS OF PLANT AND NODULE SAMPLES

i. Nitrogen:

Nitrogen was determined spectrophotometrically as described by **Nicholas and Nelsen (1957)** with slight modification of Nessler's reagent (dissolved 22 – 72 g) of $HgCl_2$ in approximately 35 ml of solution containing 18.26 kg KI. The potassium mercuric-iodide was slowly added to 980 ml of NaOH solution containing 40 g of NaOH with constant stirring. Cooled solution was made upto one litre.

Each of the samples weighed to 1 g was taken in a digestion tube and 2 ml conc. H_2SO_4 (A. R.) was added warming the tube

gently on a hot plate. 2 ml of H_2O_2 was added to each tube and they were kept overnight. On the next day, heating the tubes one by one along with drop-wise addition of H_2O_2 and the digestion was continued till the digest became colourless. A blank digestion was also carried out without adding plant or nodule sample. The digested material, after making up its volume to 100 ml was used for nitrogen estimation.

One ml of aliquot was taken in a 0.50 ml volumetric flask. After neutralising it with 2N NaOH, 1 ml Nessler's reagent was added and the volume was made up to the mark. The colour intensity was measured at 535 nm with a spectrophotometer. The sample reading was then compared against the reading of standard curve which was prepared by using different concentrations of $(NH_4)_2SO_4$ solution (dissolved 0.4712 g of pure dry ammonium sulphate in water and added 5 ml H_2SO_4 to avoid bacterial action). The solution was diluted to 100 ml.

ii. Phosphorus:

For phosphorus determination, 5 g samples taken in separate digestion tubes, were digested in the presence of 10 ml tri-acid mixture (HNO_3, $HClO_4$ and H_2SO_4) in the proportion of 10:4:1. Digest of each tube was made up to 100 ml with distilled water in volumetric flasks

Phosphorus was determined by Barton's nitric acid, ammonium molybdate reagent method as described by **Kanwar and Chopra (1976)**. 10 ml digested solution was taken into 50 ml volumetric flask to which 10 ml ammonium molybdate reagent was added and the volume was made up to 50 ml with distilled water. The intensity of the colour development was measured with Spectrophotometer at 470 nm. A blank sample was also simultaneously prepared for correction of the reading.

SEED AND STRAW ANALYSIS

i. Crude Protein /Nitrogen:

Digestion of powdered / ground seed and straw samples (1 g each) and estimation of nitrogen in the digest material were the same as in case of plant / nodule nitrogen determination.

Nitrogen percent in seeds were multiplied by a factor of 6.25 to get the crude protein percent.

ii. Phosphorus:

The ammonium molybdate reagent method, as described by **Kanwar and Chopra (1976)** was employed for the determination of phosphorus.

UPTAKE OF NUTRIENTS AND PHOSPHORUS BY GRAIN, STRAW AND THE WHOLE CROP

Plot-wise uptake of N and P by grain or straw was calculated by multiplying the plot-wise percent value of individual nutrients with the respective grain and straw yield of the same plot. Per plot uptake of individual nutrients by grain and straw were added together to estimate the plot-wise total uptake of nutrients by the crop. Uptake of nutrients has been expressed on the basis of mg/100 g.

STATISTICAL ANALYSIS

In order to draw reliable conclusion, the experimental data in relation of soil, plant, nodule and seed characteristics were analysed statistically by adopting the procedures described by Cox and Cochran (1957). Data pertaining to the observed parameters of the first year and second year experiments for the purpose of statistical analysis. Significance of treatment effect was judged against 'F' value at 5% level of significance. C.D. at 5% was calculated to the test of significance of treatment effect.

EXPERIMENTAL DETAILS

EXPERIMENT FOR THE YEAR 2000 – 2001

Treatment combination of Pea (*Pisum sativum* L.)
Variety AZAD P_1

Sl. No.	Treatment	Abbreviation
1.	Control	T_1
2.	Rhizobium	T_2
3.	Mussoorie Rock Phosphate (M R P)	T_3
4.	M R P + Rhizobium	T_4
5.	Azospirillum	T_5
6.	M R P + Azospirillum	T_6
7.	Phosphate soluble micro-organisms (P S M)	T_7
8.	M R P + P S M	T_8

Treatment combination of Wheat (*Triticum aestivum*) crop
Variety SONALIKA

Sl. No.	Treatment	Abbreviation
1.	Control	T_1
2.	Azotobacter	T_2
3.	Mussoorie Rock Phosphate (M R P)	T_3
4.	M R P + Azotobacter	T_4
5.	Azospirillum	T_5
6.	M R P + Azospirillum	T_6
7.	Phosphate soluble micro-organisms (P S M)	T_7
8.	M R P + P S M	T_8

EXPERIMENT FOR THE YEAR 2001 – 2002

Treatment combination of Pea (*Pisum sativum* L.)

Variety AZAD P_1

Sl. No.	Treatment	Abbreviation
1.	Control	T_1
2.	Rhizobium	T_2
3.	Mussoorie Rock Phosphate (M R P)	T_3
4.	M R P + Rhizobium	T_4
5.	Azospirillum	T_5
6.	M R P + Azospirillum	T_6
7.	Phosphate soluble micro-organisms (P S M)	T_7
8.	M R P + P S M	T_8

Treatment combination of Wheat (*Triticum aestivum*) crop

Variety SONALIKA

Sl. No.	Treatment	Abbreviation
1.	Control	T_1
2.	Azotobacter	T_2
3.	Mussoorie Rock Phosphate (M R P)	T_3
4.	M R P + Azotobacter	T_4
5.	Azospirillum	T_5
6.	M R P + Azospirillum	T_6
7.	Phosphate soluble micro-organisms (P S M)	T_7
8.	M R P + P S M	T_8

CHAPTER – IV

RESULT

AND

DISCUSSION

RESULT AND DISCUSSION

WHEAT

A field trial was conducted with 8 treatments in Randomised Block Design to find out the response of N-fixing and Phosphate solubilizing micro-organisms in soil and plants of wheat (*Triticum aestivum*) var. Sonalika seeds at the Crop Research Unit of Sheila Dhar Institute, during Rabi season 2000 – 2001 and 2001 – 2002 with Azotobacter, Mussoorie Rock Phosphate (MRP), Azospirillum and Phosphate Solubilizing Micro-organisms (PSM) combinations.

Plant height (cm):

The growth of wheat crop was studied at 30, 60 and 90 days after sowing (DAS), which were recorded in table 4.1.1 and depicted in figure 4.1.1.

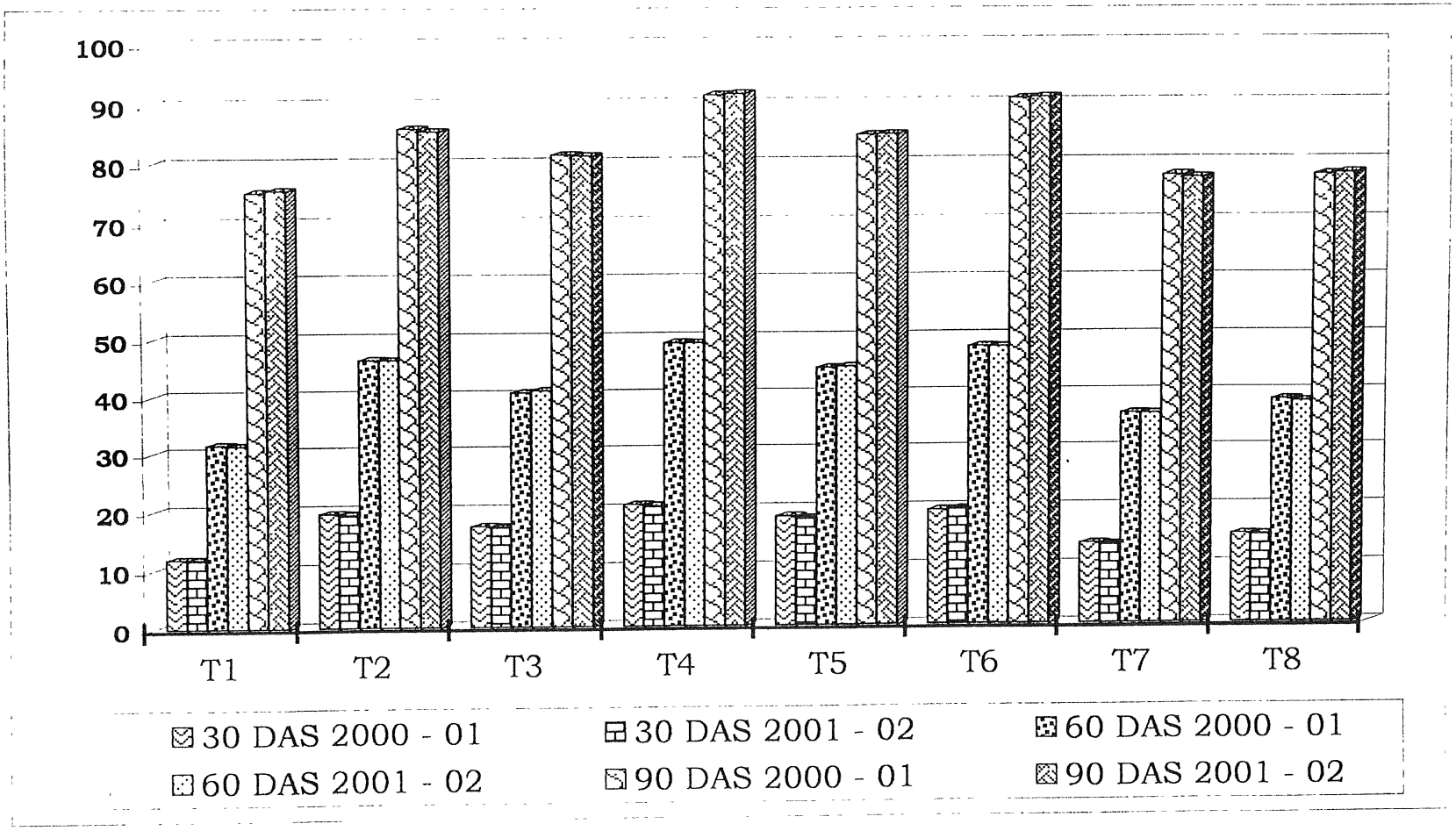
Maximum plant height at 30 DAS was recorded with M R P + Azotobacter treatment combination in both the experimental years, i.e. 2000 – 2001 and 2001 – 2002, which was 20.90 and 20.67 cm respectively, while the minimum plant height was recorded in control set in both the experimental years, which was 11.93 and 11.90 cm respectively.

Table 4.1.1 **Average plant height (cm) of Wheat as influenced by different treatments at different growth stages**

Treatments	2000 – 2001			2001 – 2002		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	11.93	31.50	75.33	11.90	31.33	75.67
T_2	19.50	46.33	85.93	19.37	46.30	85.50
T_3	17.27	40.50	81.50	17.20	40.87	81.33
T_4	20.90	49.20	91.50	20.67	49.17	91.67
T_5	18.73	44.70	84.73	18.40	44.90	84.83
T_6	19.67	48.33	90.77	19.90	48.23	91.00
T_7	13.73	36.43	77.93	13.57	36.33	77.50
T_8	15.27	38.73	78.00	15.13	38.40	78.20
CD at 5%	0.540	0.770	0.756	0.775	1.012	0.995

The plant height of wheat crop at 60 days after sowing was recorded during the experimental year 2000 – 2001 and 2001 – 2002 as 31.50 cm and 31.33 cm respectively in control. When M R P + Azotobacter was applied the crop height increased in both the years to 49.20 and 49.17 cm respectively followed by M R P + Azospirillum treatment combination in both the years of experiment as 48.33 and 48.23 cm respectively.

Fig. 4.1.1 Average plant height (cm) as influenced by different treatments at different growth stages



The height of the wheat crop at 90 DAS was recorded as 75.67 and 75.33 cm during the year 2000 - 2001 and 2001 - 2002 respectively in control. When M R P + Azotobacter was applied, the crop height increased to 91.50 and 91.67 cm respectively, followed by M R P + Azospirillum, which was recorded as 90.77 and 91.00 cm in both the years respectively. Thus, it reveals that M R P and Azotobacter treatment combination had a significant influence on the plant height.

It is interesting to note that the plant height were higher in all the three stages of growth with M R P + Azotobacter treatment. The probable reason of this could have been the better supply of nutrients and its proper utilisation by plant, which played a significant role in better plant growth and development. This resulted in maximum plant height at all the three growth stages. Azotobacter also played a vital role to control certain fungal diseases (Berezova *et al.*, 1938) and higher percentage of healthy seedling (Naumova, 1939). Rao and Sharma (1981) and Prasad et al. (1991) also reported similar findings.

Average yield attributes:

The grain yield and straw yield of wheat crop (q/ha) during the experimental years 2000–2001 and 2001–2002 have been presented in table 4.1.2 and depicted in figure 4.1.2 to 4.1.5.

Table 4.1.2 **Average Grain Yield and Straw Yield of Wheat (q/ha) as influenced by different treatments at different growth stages**

Treatments	2000 – 2001			2001 – 2002		
	Grain yield (q/ha)	Straw yield (q/ha)	Grain – straw ratio	Grain yield (q/ha)	Straw yield (q/ha)	Grain – straw ratio
T_1	20.50	38.27	1.83	20.33	37.41	1.84
T_2	28.50	40.33	1.70	28.67	49.02	1.71
T_3	24.67	52.00	1.67	24.90	41.58	1.67
T_4	30.67	45.93	1.77	30.87	54.33	1.75
T_5	27.50	52.67	1.67	27.27	45.26	1.70
T_6	28.33	47.60	1.73	28.63	49.39	1.73
T_7	21.67	44.27	1.90	21.83	41.27	1.90
T_8	24.50	47.50	1.87	24.43	45.69	1.87
CD at 5%	0.863	1.049	0.144	0.873	1.539	0.041

The minimum grain yield during the experimental year 2000-2001 (Fig. 4.1.2) and 2001-2002 (Fig. 4.1.3) was recorded with control, i.e. 20.50 and 20.35 q/ha, respectively. Maximum grain yield of wheat during the year 2000-2001 and 2001-2002 was observed with M R P + Azotobacter treatment combination, i.e. 30.67 and 30.87 q/ha respectively, followed by M R P + Azospirillum treatment combination, i.e. 28.83 and 28.63 q/ha respectively. The probable

Fig. 4.1.2 Average grain yield (q/ha) : 2000 - 2001

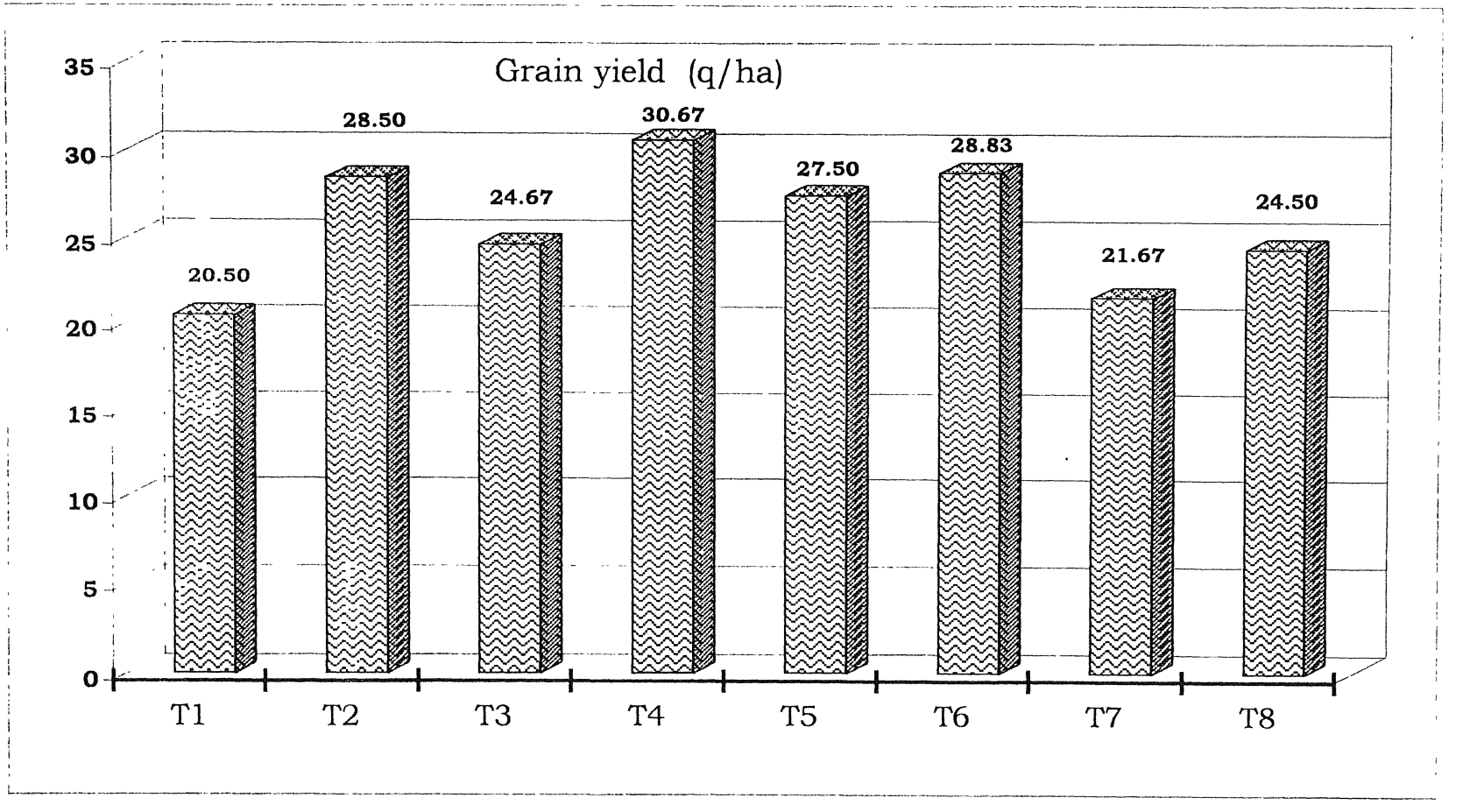


Fig. 4.1.3 Average grain yield (q/ha) : 2001 - 2002

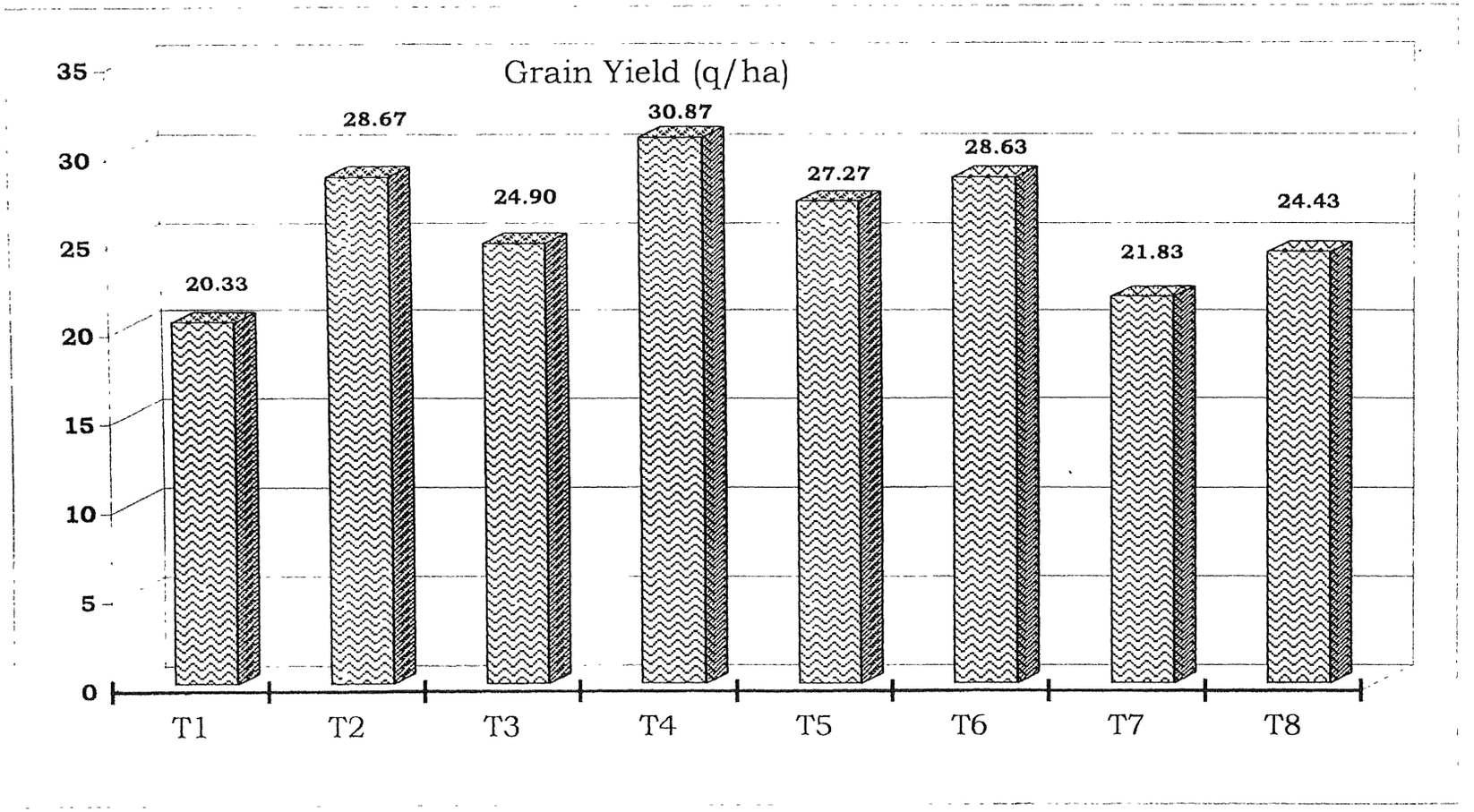


Fig. 4.1.4 Average straw yield (q/ha) : 2000 - 2001

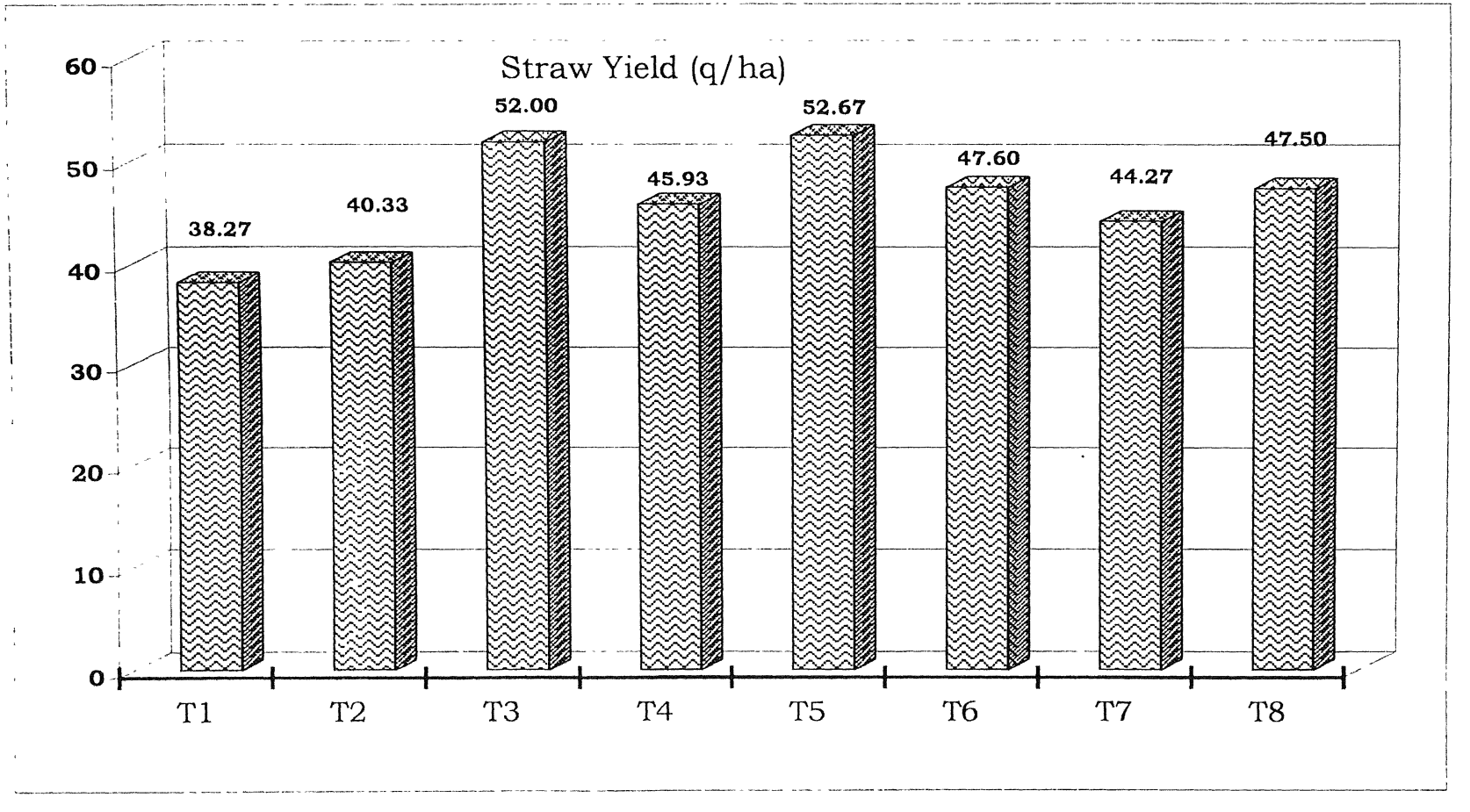
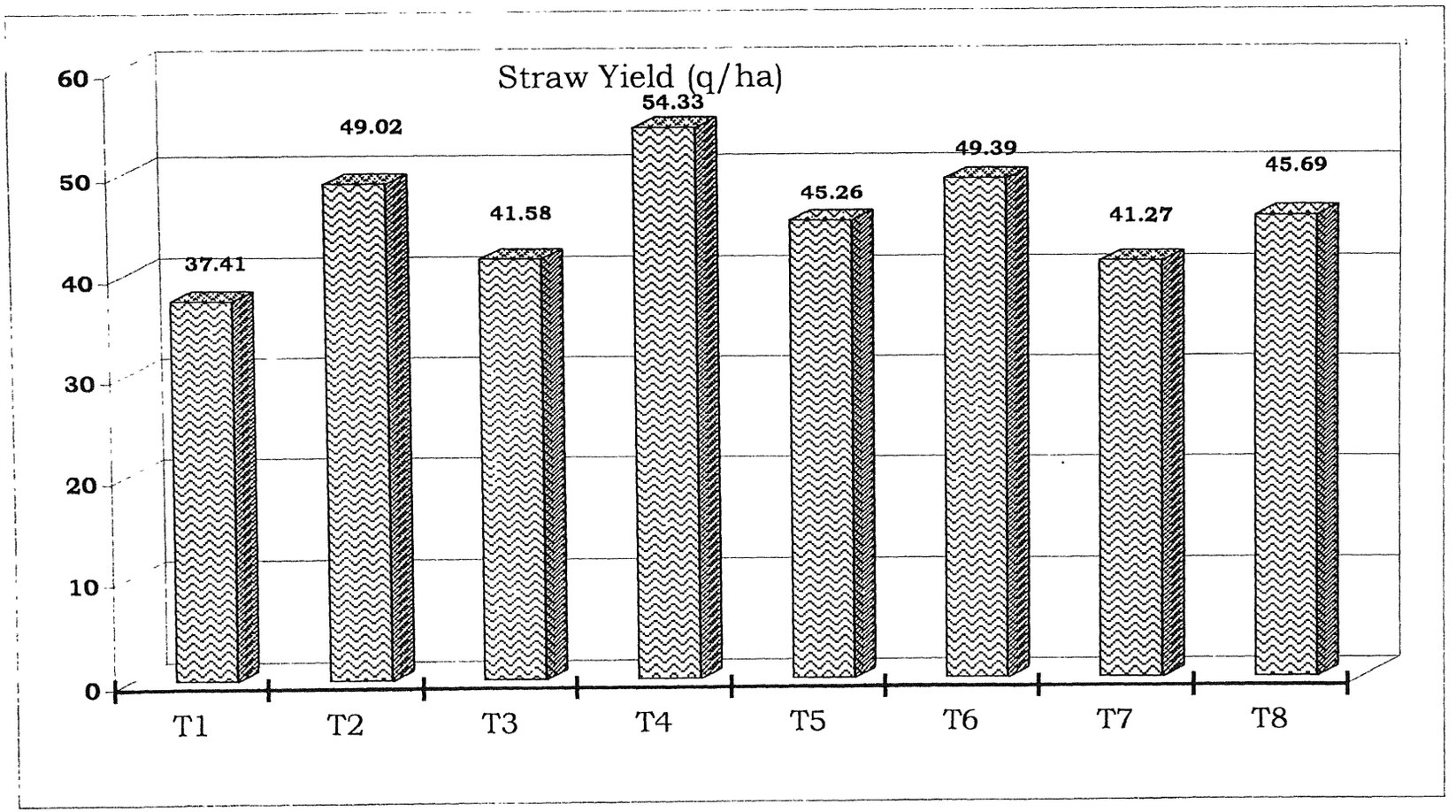


Fig. 4.1.5 Average straw yield (q/ha) : 2001 - 2002



reason for this may be due to increased availability of phosphates. **Kulikovskaya (1954–55)** reported that due to Azotobacter treatment the yield increased by 34.2 to 37.5 percent higher than the control plants. **Rosario and Barea (1975)** have reported similar results. **Vasakhnil and Manorik (1954)** and **Yung (1954)** also reported that application of Azotobacter and Rock phosphate gave significantly higher grain yield.

The maximum straw yield was recorded with Azospirillum treatment combination in the year 2000-2001 (Fig. 4.1.4), which was 52.67 q/ha. In the year 2001-2002 (Fig. 4.1.5), similar trend was observed, as in case of grain yield, i.e. 54.33 q/ha. The minimum straw yield was recorded with control set, in both the experimental years, i.e., 2000-2001 and 2001-2002, which was 38.27 and 37.41 q/ha, respectively. The probable reason for such findings could be due to supply of more nitrogen, which increased the growth of the plant, especially the plant height. **Kumaran *et al.* (1998)** reported that Azospirillum and phosphobacteria gave significant response in terms of growth and yield and plant height.

Nitrogen and Phosphorus uptake by wheat grain:

Wheat grains were analysed for their N and P contents and the observations are presented in Table 4.1.3 and depicted in figure 4.1.6 and 4.1.7.

Table 4.1.3 **Average N-uptake and P-uptake of wheat grain as influenced by different treatment combinations**

Treatments	2000 – 2001			2001 – 2002		
	N-uptake in grain (kg/ha)	P-uptake in grain (kg/ha)	N-P uptake ratio	N-uptake in grain (kg/ha)	P-uptake in grain (kg/ha)	N-P uptake ratio
T_1	1.747	0.034	49.50	1.749	0.035	49.52
T_2	1.807	0.035	49.83	1.815	0.036	49.85
T_3	2.031	0.044	46.30	2.023	0.034	46.40
T_4	0.080	0.039	53.60	2.082	0.039	53.64
T_5	2.061	0.038	52.77	2.062	0.039	53.43
T_6	2.171	0.047	46.30	2.170	0.047	46.62
T_7	1.820	0.036	50.27	1.822	0.037	49.52
T_8	2.018	0.047	41.17	2.019	0.049	41.40
CD at 5%	0.008	0.002	0.567	0.012	0.002	2.171

On perusal of data contained in table 4.1.3 and figure 4.1.6, it was noticed that N-uptake in wheat grain was recorded as 1.747 and 1.749 kg/ha during the experimental year 2000-2001 and 2001-2002 respectively, in the control set. When the crop was incorporated with M R P + Azotobacter treatment combination, in both the experimental years, N-uptake was 2.080 and 2.082 kg/ha respectively. It was observed that the application of M R P + Azotobacter increased not

Fig. 4.1.6 Nitrogen uptake in wheat grain and wheat straw (kg/ha) as influenced by different treatments

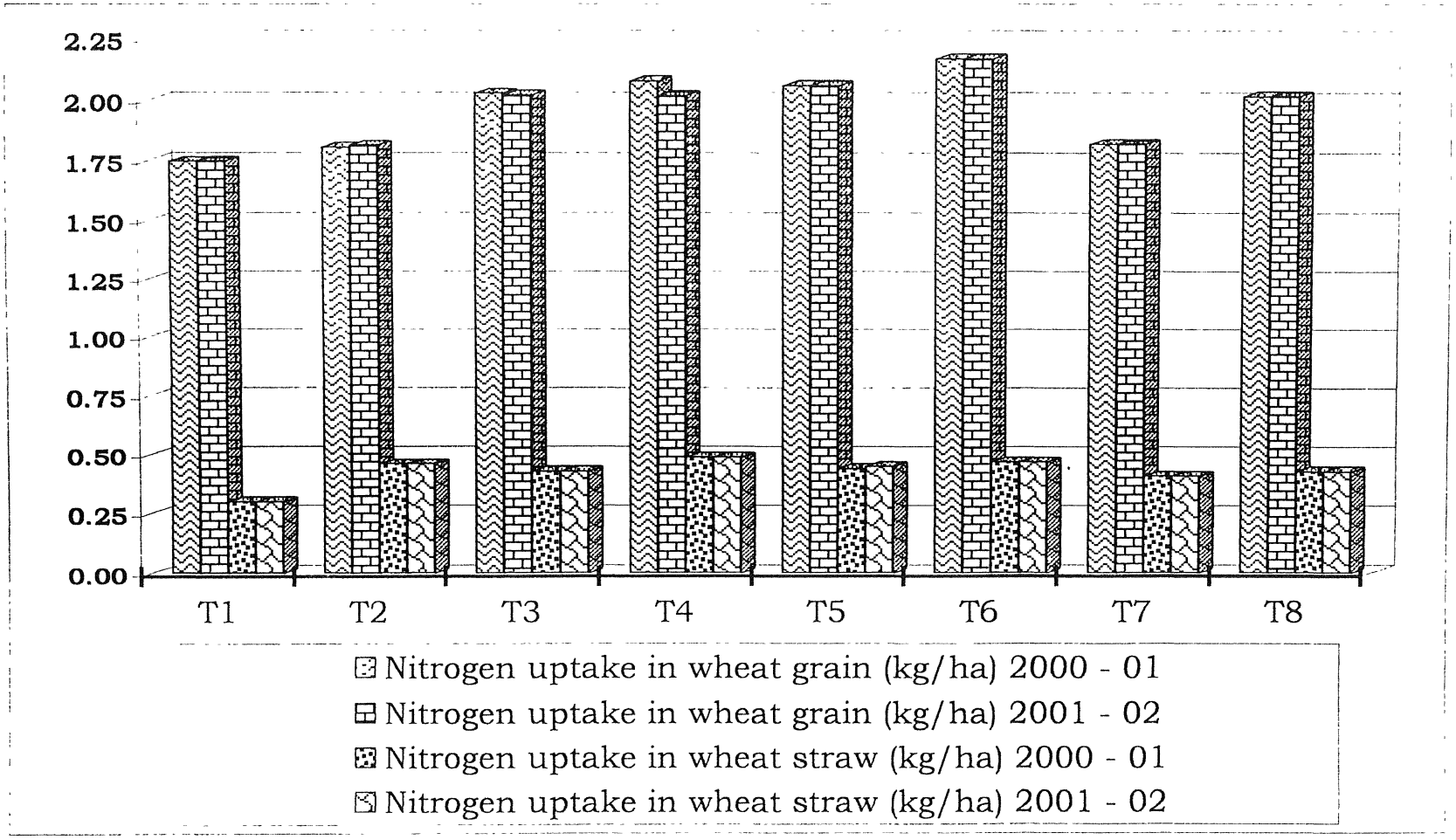
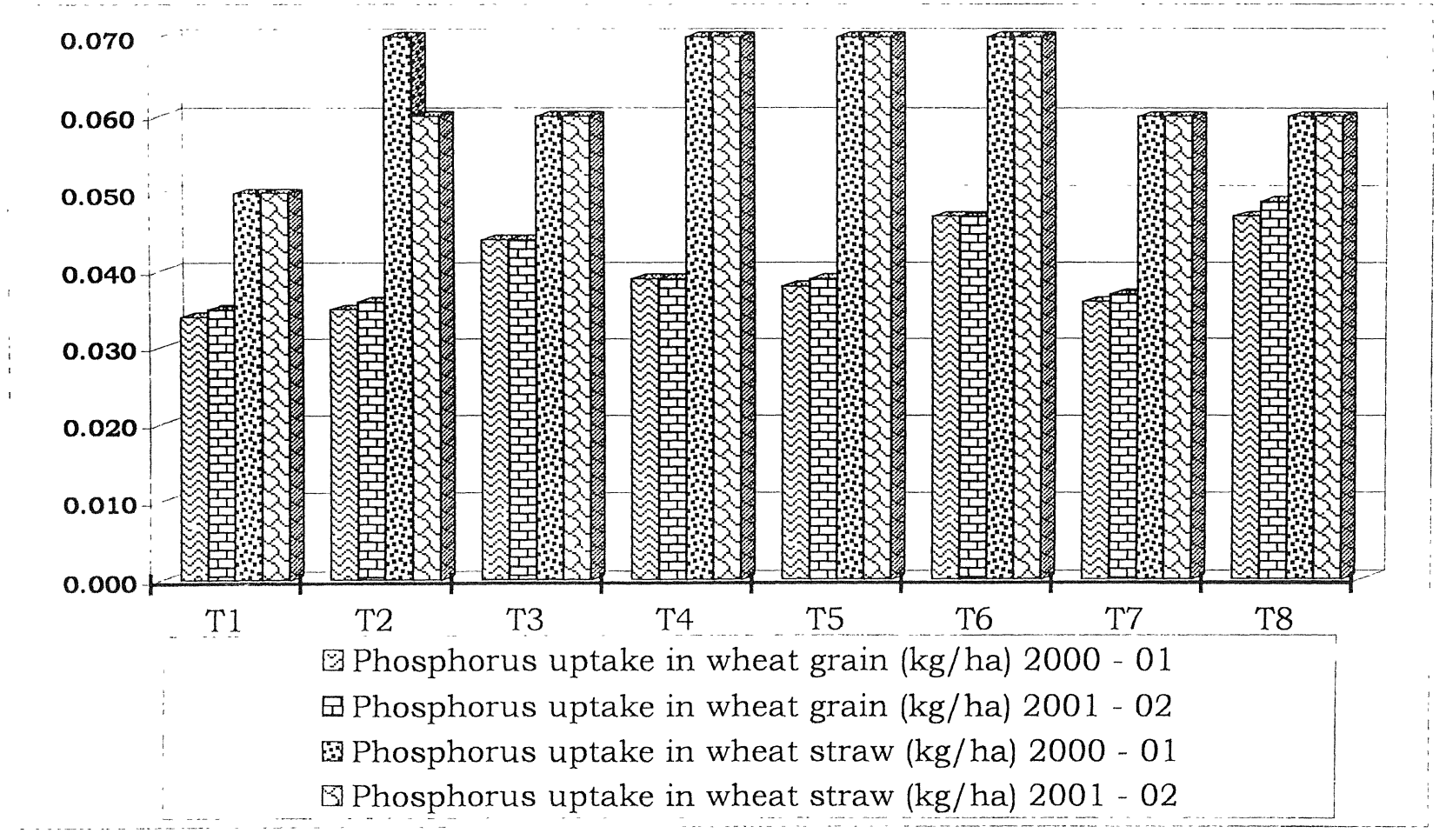


Fig. 4.1.7 Phosphorus uptake in wheat grain and wheat straw (kg/ha) as influenced by different treatments



only crop yield but also its nutrient content, especially nitrogen. The increased N-uptake by wheat grain may be due to availability of more nitrogen. **Klivos'ka (1954–55)** reported that Azotobacter was effective in significantly increasing the nitrogen content of wheat grain.

The grains were also analysed for their P-uptake and the findings have been presented in Table 4:1.3 and graphically depicted in figure 4.1.7.

The P-uptake in grain was estimated as 0.034 and 0.035 kg/ha in the experimental years 2000-2001 and 2001-2002, respectively, in control set. With the application of M R P, the P-content got slightly increased to 0.44 kg/ha in both the years of experimentation. This may be due to increased amount of available P_2O_5 through M R P.

Nitrogen and Phosphorus uptake by wheat straw:

The N and P content in wheat straw were estimated and presented in Table 4.1.4 and graphically depicted in figure 4.1.6 and 4.1.7.

On perusal of the data contained in table 4.1.4, it may be noticed that the nitrogen uptake by wheat straw in control plots was recorded as 0.30 kg/ha during both the experimental years, i.e. 2000-2001 and 2001-2002.

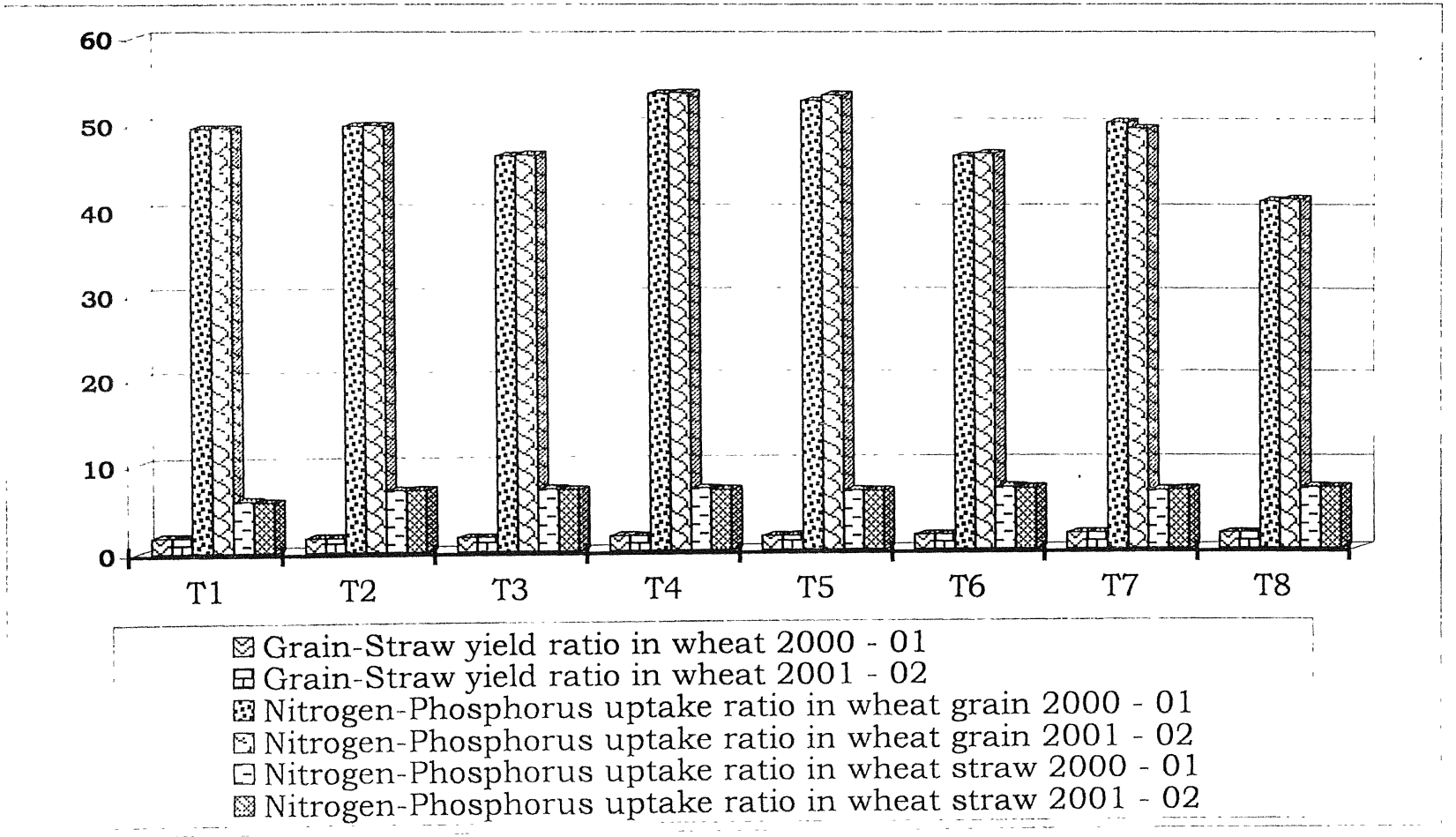
Table 4.1.4 **Average N-uptake and P-uptake of wheat straw as influenced by different treatment combinations**

Treatments	2000 – 2001			2001 – 2002		
	N-uptake in straw (kg/ha)	P-uptake in straw (kg/ha)	N-P uptake ratio	N-uptake in straw (kg/ha)	P-uptake in straw (kg/ha)	N-P uptake ratio
T_1	0.30	0.05	5.95	0.30	0.05	5.77
T_2	0.46	0.07	7.04	0.46	0.06	7.08
T_3	0.43	0.06	7.14	0.43	0.06	7.06
T_4	0.49	0.07	7.10	0.49	0.07	6.95
T_5	0.44	0.07	6.82	0.45	0.07	6.77
T_6	0.47	0.07	7.14	0.47	0.07	7.02
T_7	0.41	0.06	6.76	0.41	0.06	6.82
T_8	0.43	0.06	7.07	0.43	0.06	7.05
CD at 5%	0.009	0.001	0.175	0.010	0.001	0.007

The N-uptake by straw got increased to 0.49 kg/ha in both the experimental years, with M R P + Azotobacter treatment combination, followed by 0.47 kg/ha with M R P + Azospirillum treatment combination during both the years of experimentation.

A similar trend in the increase of P-uptake by wheat straw was obtained as influenced by different treatment combination, as was observed in case of wheat grain.

Fig. 4.1.8 Average yield attributes of wheat as influenced by different treatments



The explanation for increased N and P uptake by straw may be due to absorption of higher amount of N and P by the plant roots, resulting in higher N and P uptake by the straw, as influenced by the treatment combination M R P + Azotobacter followed by M R P + Azospirillum. Sanoria and Rao (1973 - 74) stated on their experiment that Azotobacter was effective in significantly increasing the N content of wheat grain and straw when the soil was basal dressed with super phosphate.

Grain – straw ratio:

The data relating to ratio between wheat grain and wheat straw; N and P uptake by wheat grain; and N and P uptake by wheat straw are presented in tables 4.1.2, 4.1.3 and 4.1.4 respectively. These ratios have also been graphically depicted in figure 4.1.8.

Perusal of the aforementioned tables and figure would reveal the similar trend of influence of different treatment combinations as observed in case of growth and yield attributes of wheat crop.

PEA

A field trial was conducted with 8 treatments in Randomised Block Design to find out the response of N-fixing and Phosphate solubilizing micro-organisms in soil and plants of Pea (*Pisum sativum* L.) var. Azad P_1 seeds at the Crop Research Unit of Sheila Dhar

Institute, during Rabi season 2000 – 2001 and 2001 – 2002 with Rhizobium, Mussoorie Rock Phosphate (MRP), Azospirillum and Phosphate Solubilizing Micro-organisms (PSM) combinations.

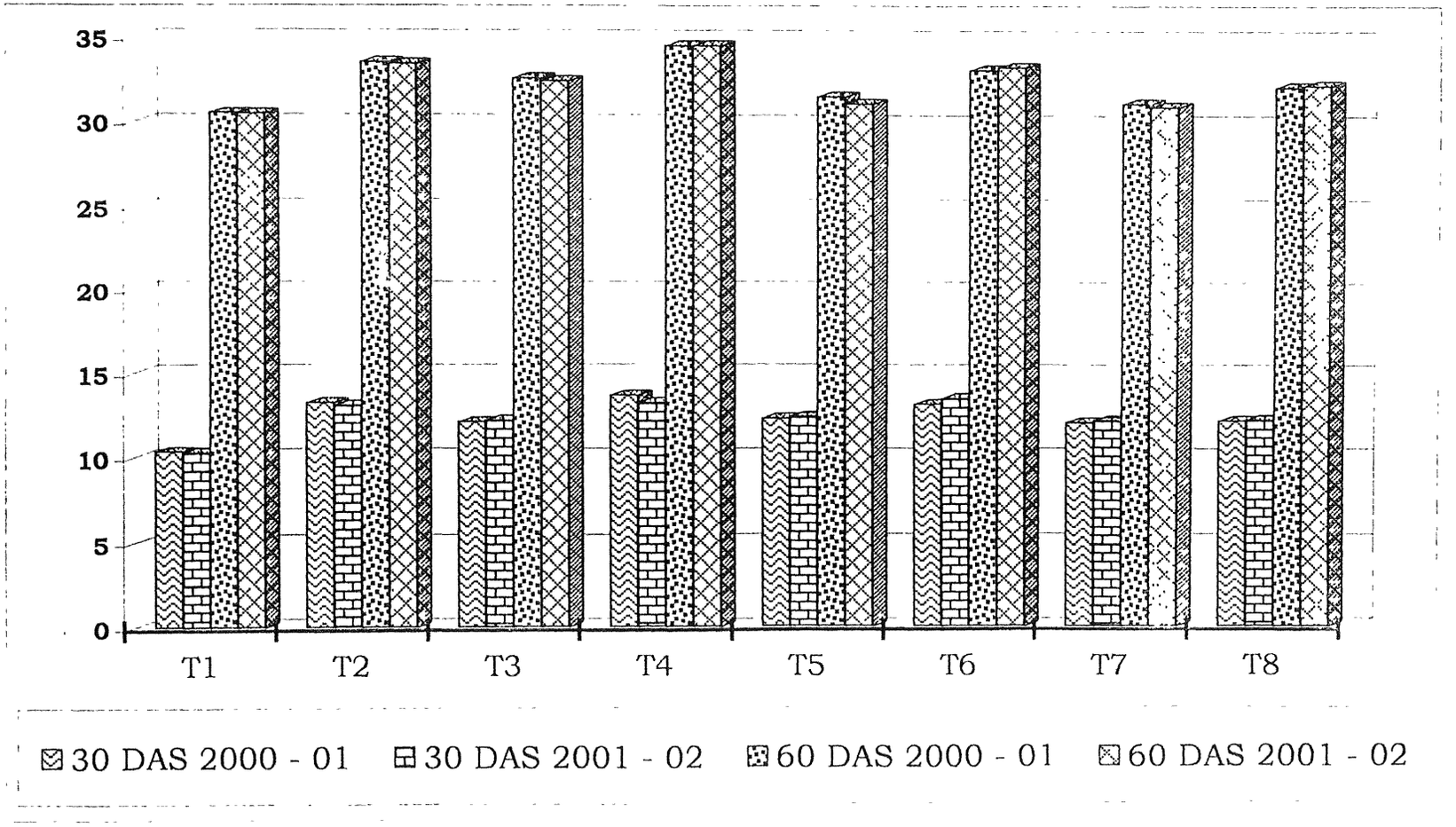
Plant height (cm):

The plant height at the active growth stages and at pod filling stage as influenced by different biofertilizers are presented in table 4.2.1 and depicted in figure 4.2.1.

Table 4.2.1 Average plant height of Pea (cm) as influenced by different treatments at different growth stages

Treatments	2000 – 2001		2001 – 2002	
	30 DAS	60 DAS	30 DAS	60 DAS
T_1	10.33	30.57	10.28	30.53
T_2	13.27	33.60	13.10	33.50
T_3	12.10	32.67	12.20	32.50
T_4	13.70	34.57	13.23	34.57
T_5	12.03	31.60	12.40	31.17
T_6	13.13	33.17	13.50	33.33
T_7	12.03	31.20	12.17	31.00
T_8	12.17	32.20	12.23	32.30
CD at 5%	0.238	0.513	0.628	1.086

Fig.4.2.1 Average plant height (cm) as influenced by different treatments at different growth stages



The minimum plant height at both the stages during both the years of experimentation, i.e. 2000 – 2001 and 2001 – 2002 was observed with control set. At 30 DAS, the plant height was recorded as 10.33 cm in 2000 – 2001 and 10.28 cm in 2001 – 2002. At the active growth stage it was 30.57 cm and 30.53 cm respectively, at pod filling stage (60 DAS).

On examination of the data, maximum plant height was observed with M R P + Rhizobium treatment combination in both the experimental years, at 30 days after sowing (DAS), i.e. 13.70 and 13.23 cm during 2000-2001 and 2001-2002, respectively. The plant height at 60 DAS was recorded as 34.57 cm during both the years of experimentation. The probable reason for the these results could be due to the growth regulators produced by Rhizobium and M R P and also might be due to the solubilization of insoluble phosphates by production of various organic acids.

Bhandal *et al.* (1989) reported that inoculation by Rhizobium had significant effect on plant height and dry matter accumulation at pod filling stage and at flowering stage in pea. **Alagawadi and Gaur (1998)** had also given similar report to confirm this type of findings.

Grain yield (q/ha):

The grain yield (q/ha) of pea was recorded after threshing. The values are presented in table 4.2.2 and depicted in figure 4.2.2 and 4.2.3.

Table 4.2.2 Average grain yield of Pea (q/ha) as influenced by different treatments

Treatments	2000 – 2001	2001 – 2002
	Grain yield (q/ha)	Grain yield (q/ha)
T_1	40.33	40.67
T_2	61.00	61.17
T_3	54.00	54.33
T_4	61.00	61.20
T_5	43.33	43.53
T_6	56.20	55.87
T_7	50.33	50.67
T_8	52.17	52.10
CD at 5%	1.345	1.380

An observation of the data contained in table 4.2.2 indicates that the maximum grain yield was observed with M R P + Rhizobium treatment combination in both the years of experimentation. During

Fig. 4.2.2 Average yield (q/ha) : 2000 - 2001

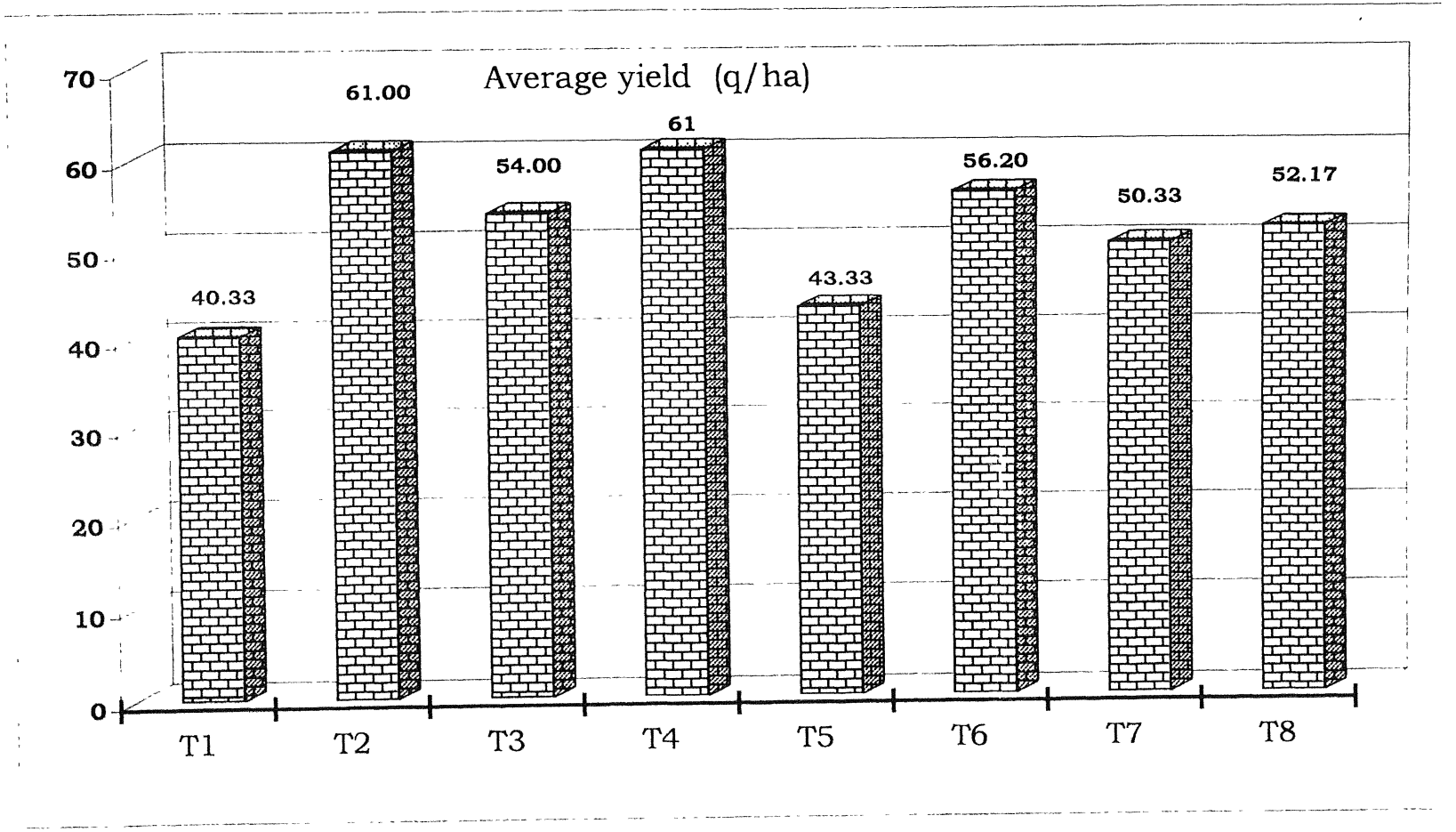
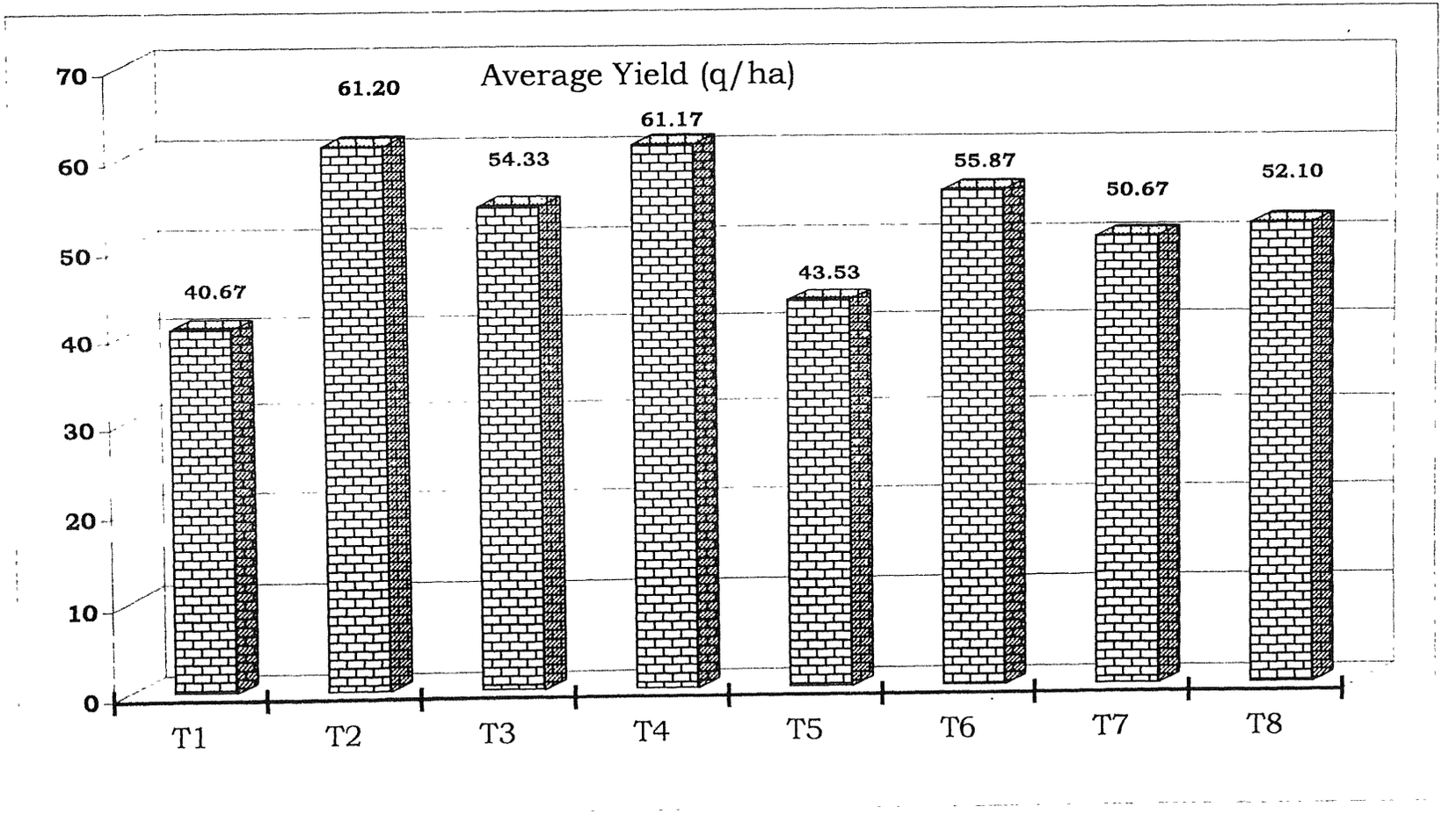


Fig. 4.2.3 Average yield (q/ha) : 2001 - 2002



the first year of experiment, i.e. 2000–2001 (Fig. 4.2.2) the average grain yield was 61.00 q/ha whereas in the second year, i.e. 2001–2002 (Fig. 4.2.3) the grain yield was 61.20 q/ha, followed by Rhizobium treated plots, which was 61.00 and 61.17 q/ha, during the first and second year, respectively. The minimum grain yield was observed with the control set, which was 40.33 and 40.67 q/ha in 2000 – 2001 and 2001 – 2002 respectively.

Over-viewing the performance of grain yield (q/ha), M R P + Rhizobium played a vital role in boosting the plant growth because of availability of nitrogen, proper grain filling and other yield attributes which subsequently increased the grain yield. **Haque *et al.* (1988); Jain, Joshi and Taneja (1988)** and **Surendra *et al.* (1993)** had given the similar reports.

Yield attributes:

Effect of biofertilizer application on number of pods per plant, length of pod (cm), number of grains per pod and number of nodules have also been recorded and the relevant data is presented in table 4.2.3 and graphically depicted in figure 4.2.4.

A slight difference was found in the number of pods per plant, under different treatment combinations. The maximum number of pods per plant was observed with M R P + Rhizobium treatment

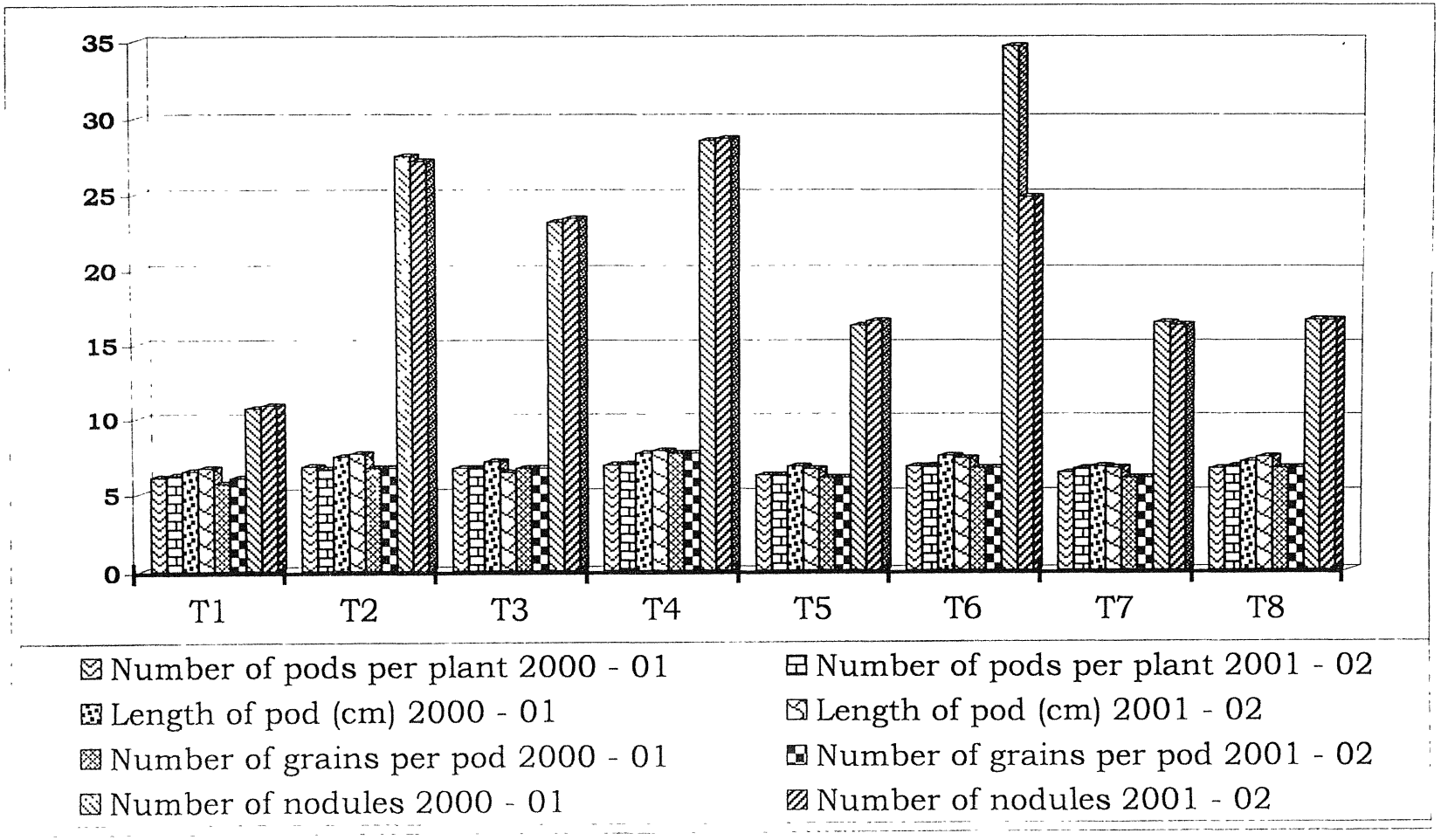
combination in both the years of experimentation, i.e. 2000 – 2001 and 2001 – 2002, which was 6.87 and 6.97 respectively. The minimum number of pods per plant were counted with the control set, in both the years, which was 6.03 and 6.17 respectively.

Table 4.2.3 Average yield attributes of pea as influenced by different treatments

Treat-ments	No. of pods per plant		Length of pod (cm)		No. of grains per pod		No. of nodules	
	2000-2001	2001-2002	2000-2001	2001-2002	2000-2001	2001-2002	2000-2001	2001-2002
T_1	6.03	6.17	6.47	6.67	5.67	6.00	10.67	10.87
T_2	6.77	6.60	7.43	7.63	6.67	6.67	27.53	27.20
T_3	6.67	6.67	7.10	7.37	6.67	6.67	23.17	23.43
T_4	6.87	6.97	7.67	7.83	7.67	7.67	28.53	28.67
T_5	6.17	6.17	6.77	6.60	6.00	6.00	16.27	16.60
T_6	6.77	6.80	7.47	7.30	6.67	6.67	24.67	24.83
T_7	6.37	6.63	6.77	6.67	6.00	6.00	16.50	16.33
T_8	6.67	6.77	7.10	7.43	6.67	6.67	16.67	16.67
CD at 5%	0.156	0.476	0.320	0.706	0.844	NS	0.741	1.176

Similar trend was found for length of pods and number of grains per pod under different treatment combinations.

Fig. 4.2.4 Average yield attributes as influenced by different treatments



Number of nodules were counted at pod filling stage. The maximum number of nodules was observed with M R P + Rhizobium treatment combination in both the experimental years, which were 28.53 and 28.67 per plant respectively, while the minimum number of nodules were counted with control set which was 10.67 and 10.87 respectively in the first and second year of experimentation, i.e. 2000 – 2001 and 2001 – 2002. This type of findings may be due to application of Rhizobia bacteria because the Rhizobium can fix lot of atmospheric nitrogen. **Singh, 1984; Sardina *et al.*, 1986; Gaur and Gaind, 1993; Reddy and Reddy, 1995** have also reported the effect of inoculation of Rhizobium on yield attributes of leguminous crop.

Fresh weight of leaf (g):

The fresh weight of leaves (g) at pod filling stage as influenced by different biofertilizers, fresh weight (g) of shoot and root system at termination of the experiment were also recorded and the relevant data are presented in table 4.2.4 and graphically depicted in figure 4.2.5.

The minimum fresh weight of leaves was observed in control set in both the years of experimentation, which was 41.50 g and 41.20 g in 2000 – 2001 and 2001 – 2002, respectively.

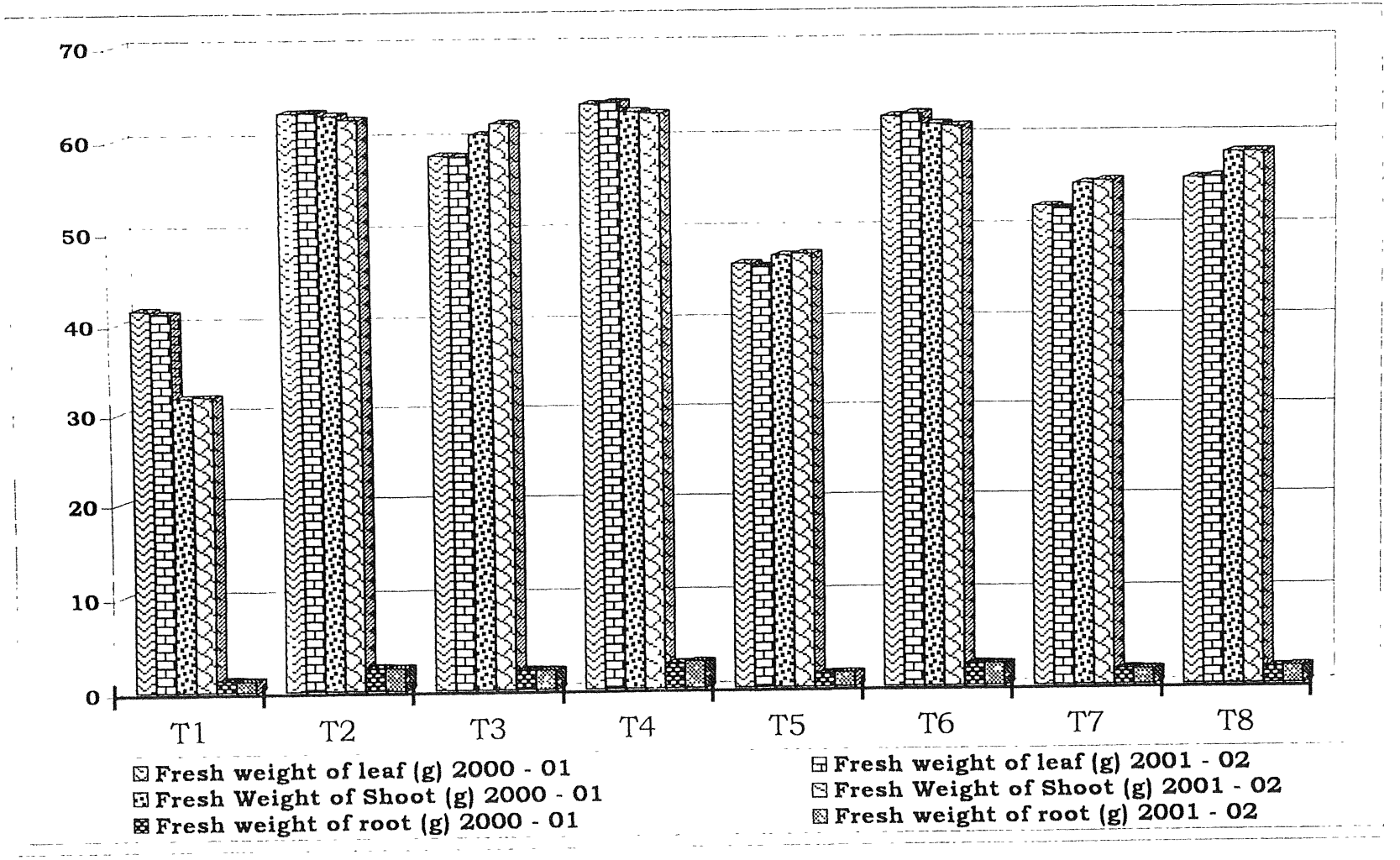
Table 4.2.4 **Average yield attributes of pea as influenced by different treatments – Fresh weight of leaves, shoot and root (g)**

Treatments	Fresh weight of Leaf (g)		Fresh weight of Shoot (g)		Fresh weight of Root (g)	
	2000-2001	2001-2002	2000-2001	2001-2002	2000-2001	2001-2002
T_1	41.50	41.20	31.74	31.80	1.23	1.20
T_2	62.87	62.97	62.60	62.17	2.47	2.43
T_3	58.23	58.20	60.50	60.67	2.13	2.10
T_4	63.67	63.87	62.83	62.63	2.70	2.83
T_5	46.33	46.00	47.17	47.33	1.47	1.50
T_6	62.17	62.43	61.33	61.10	2.37	2.33
T_7	52.33	52.07	54.83	55.10	1.70	1.63
T_8	55.30	55.50	58.17	58.20	1.77	1.87
CD at 5%	0.507	1.617	0.930	1.515	0.198	0.453

Maximum fresh weight of leaves was observed with treatment combination of M R P + Rhizobium, in both the years of experiment, which was 63.67 g and 63.87 g during the year 2000-2001 and 2001-2002, respectively.

Similar trend was found in the fresh weight of shoot and fresh weight of root system.

Fig. 4.2.5 Average yield attributes as influenced by different treatments



The probable reason for this type of findings may be due to accumulation of more nitrogen in leaves, shoot and root through the Rhizobium inoculation. **Bandopadhyay (1988)** and **Gupta and Sharma (1989)** have also reported similar results.

Dry weight of leaf (g):

The dry weight of leaves (g) as influenced by different biofertilizers, dry weight (g) of shoot and root system after termination of the experiment and complete drying of the relevant parts of the plants were also recorded and the relevant data are presented in table 4.2.5 and graphically depicted in figure 4.2.6.

The minimum dry weight of leaves was observed in control set in both the years of experimentation, which was 6.33 g and 6.53 g in 2000 – 2001 and 2001 – 2002, respectively.

Maximum dry weight of leaves was observed with M R P + Rhizobium treatment combination in both the years of experimentation, i.e. 2000 – 2001 and 2001 – 2002, which was 13.63 g and 13.67 g, respectively.

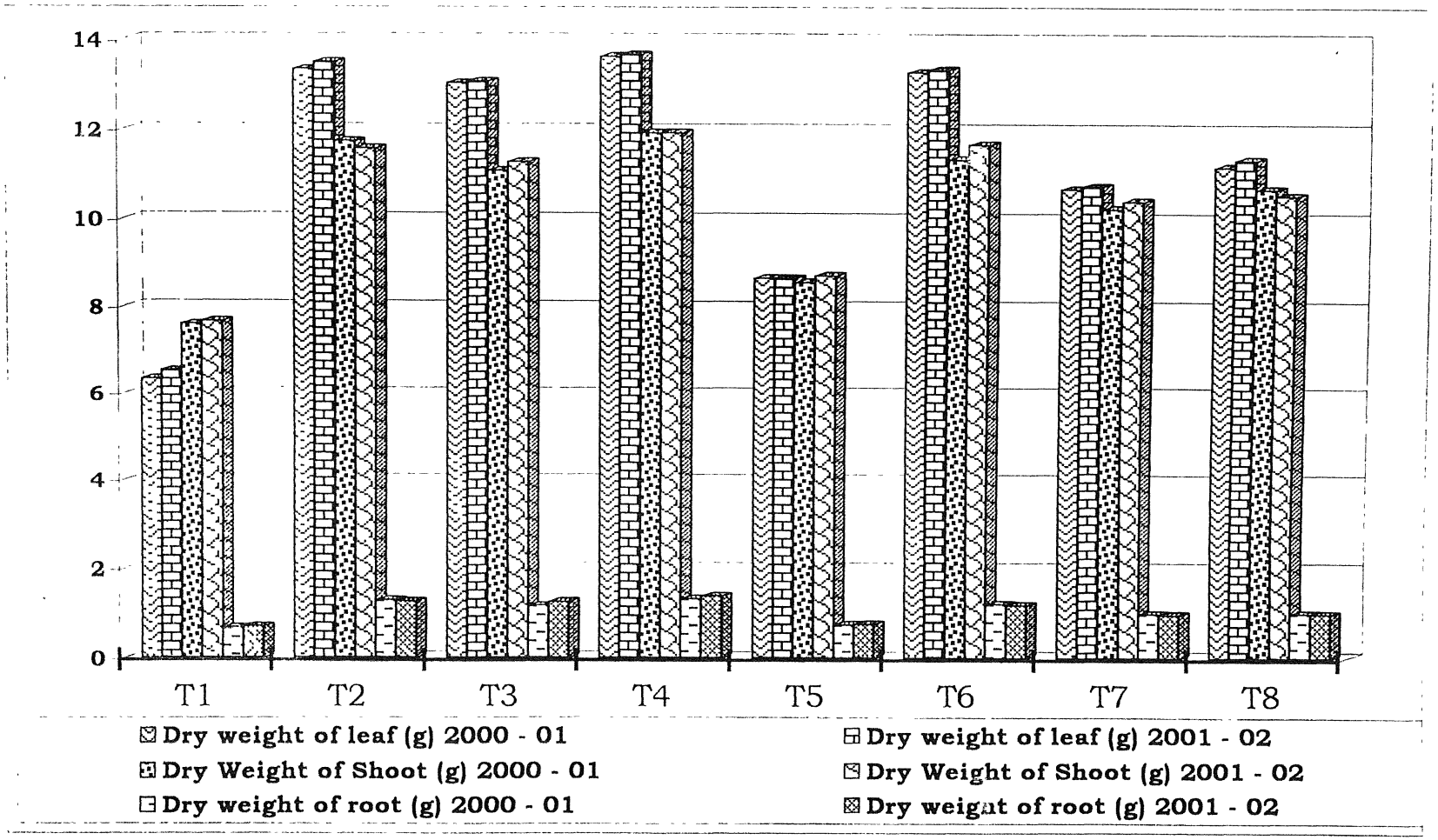
Similar findings were recorded the dry weight of shoot (g) and dry weight of root (g) in both the years of experiment, i.e. 2000 – 2001 and 2001 – 2002.

Table 4.2.5 **Average yield attributes of pea as influenced by different treatments – Dry weight of leaves, shoot and root (g)**

Treatments	Dry weight of Leaf (g)		Dry weight of Shoot (g)		Dry weight of Root (g)	
	2000-2001	2001-2002	2000-2001	2001-2002	2000-2001	2001-2002
T_1	6.33	6.53	7.60	7.67	0.67	0.69
T_2	13.33	13.50	11.73	11.57	1.28	1.26
T_3	13.03	13.07	11.07	11.27	1.16	1.25
T_4	13.63	13.67	11.90	11.93	1.32	1.38
T_5	8.67	8.67	8.57	8.73	0.74	0.75
T_6	13.27	13.33	11.33	11.67	1.20	1.17
T_7	10.67	10.73	10.23	10.40	0.98	0.96
T_8	11.17	11.33	10.67	10.53	0.99	0.98
CD at 5%	0.448	0.824	0.691	1.001	0.043	0.223

The reason of this type of findings may be due to Rhizobium inoculation because Rhizobium increases the availability of nitrogen to the plants which results into growth and development of plants. Similar reports and findings have also been given by **Nemwad (1991)** and **Kumar and Gautam (1992)**.

Fig. 4.2.6 Average yield attributes as influenced by different treatments



CHAPTER – V

SUMMARY

AND

CONCLUSION

SUMMARY AND CONCLUSION

An experiment entitled "Studies on N-fixing and P-solubilizing microorganisms in soil and plants" was conducted during Rabi season 2000 – 2001 and 2001 – 2002. Azotobacter, M R P, Azospirillum and P S M were used for pea crop. The test variety of wheat crop Sonalika and Azad P – 1 for pea was tried in both the experimental years. The experiment was conducted in Randomized Block Design having three replications, at Crop Research Unit of Sheila Dhar Institute of Soil Science.

The observations recorded during entire experimental period reveal the following summary of the findings and subsequent conclusion:

WHEAT EXPERIMENT

1. The combinations of Azotobacter, Azospirillum and M R P affect the plant height significantly in all the intervals during growth period in both the experimental years. The maximum plant height was observed with T_4 (M R P + Azotobacter) followed by T_6 (M R P + Azospirillum) in both the experimental years, while minimum was recorded with T_1 (control)

2. The combination of M R P and Azotobacter had significant effect over the grain yield (q/ha). The highest grain yield was observed with T_4 treatment combination (M R P + Azotobacter) followed by T_6 (M R P + Azospirillum) whereas the minimum was observed with T_1 (control).

3. The maximum straw yield (q/ha) was recorded with Azospirillum treatment combination in both the experimental years, i.e. 2000 – 2001 and 2001 – 2002 while the minimum straw yield was recorded with control set, that was T_1.

4. It was observed that M R P and culture Azotobacter influenced the N-uptake by wheat grain. The combination of M R P and Azotobacter significantly increased the N-uptake in wheat grain in both the experimental years while minimum was with control set. In case of P-uptake, the highest value was recorded with T_3 (M R P) while the minimum was with control set.

5. A similar trend in the increase of N-uptake and P-uptake of wheat straw was obtained as influenced by different treatment combination as was observed in case of wheat grain.

6. The grain – straw ratio of wheat crop revealed the similar trend of influence of different treatment combinations as observed in case of growth and yield attributes of wheat crop.

PEA EXPERIMENT

1. There had not been much differences over plant height in different treatment combinations at successive growth stages in both the experimental years. The highest plant height was observed with T_4 (M R P + Rhizobium) treatment combination in both the experimental years at the successive growth stages while the minimum was recorded with treatment T_1 (control).

2. The grain yield (q/ha) of pea crop was greatly influenced by different types of culture singly as well as their combinations. The maximum grain yield was recorded with T_4 (M R P + Rhizobium) treatment in both the experimental years, i.e. 2000 – 2001 and 2001 – 2002 whereas the minimum was observed with treatment T_1 (control).

3. There had been slight difference in yield attributes, such as number of pod per plant, number of grains per pod, length of pod (cm) and number of nodules per plant. The different treatments influenced marginally over the above mentioned yield attributes of pea crop. The combination of M R P + Rhizobium showed the superiority over all other treatment combinations on all the above mentioned yield attributes in

both the years of experimentation, i.e. 2000 – 2001 and 2001 – 2002.

4. The culture and their combination influenced the fresh weight (g) of pea plant. The maximum fresh weight (g) was observed with treatment T_4 (M R P + Rhizobium) in both the experimental years.

5. The dry weight of leaves (g), dry weight of shoots (g) and root system differed in different treatments. The maximum dry weight of leaves was observed with M R P + Rhizobium treatment combination in both the years while the minimum was with treatment T_1 (control). Similar trends were observed on dry weight of shoot (g) and dry weight of root (g) in both the years.

CONCLUSION

To conclude, the present study indicates that the ideal treatment combination for obtaining maximum yield of wheat is M R P + Azotobacter and M R P + Rhizobium for pea crop.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abd-EL-Malek, Y.; Hosny, I. and Shawky, B. T. 1975 Available of Soil ammonical nitrogen to Azotobacter and its effect on nitrogen fixation. Infektionskrankheitin and Hygiene II, 130 : 584 – 597. Cited from Soils and Fertilizer., 39 , 1976, 300.

Agrawal, D. and Sanoria, C. L. 1978 Preliminary testing of strains of Azotobacter chroococcum for their suitability as seed inoculant. SCINAL, 44 : 318 – 319.

Ahmed, N. and Jha, K. K. 1977 Effect of inoculation with phosphate solubilizing organisms on the yield and P-uptake of gram. J. Ind. Soc. Soil Sci., 25 (4) : 319 – 393.

Ahmed, N.; Rai, M. and Sahi, B. P. 1979 Morphological and cultural characteristics of salt and alkali tolerant strains of Azotobacter chroococcum, Curr. Sci., 48 : 321.

Alexander, M. 1977 Introduction to Soil Microbiology, John Nilley and Sons Inc., New York and London, 27 : 435.

Allison, F. and Hoovers, S. 1934 An accessory factor for legume nodule bacteria. J. Bact., 27 : 561.

Allison, F. and Minor, F. 1938 Coenzyme R. requirement of Rhizobium. Soil Sci., 46 : 443.

Apte, R. and Isqoran, V. 1974 Culture of Rhizobium inoculants with those of Beijerinckia and Azotobacter. Proc. Ind. Nat. Sci. Acad. Part B, 40 : 482 – 485.

Arora, D. 1975 Studies of rock phosphate solubilizing micro-organisms in relation to crop yields and phosphate uptake. Ph. D. Thesis, P. G. School, I. A .R. I., New Delhi.

Az Con-C-de Aguilar 1978 Effect of interactions between different culture fractions of phosphobacterin and Rhizobium on mycorrhizal infection, growth and nodulation of Medicago Sativa. Con. J. Microbiol., 24 (5) : 520 – 524.

Azad, A. S.; Gill, A. S. and Dhaliwal, H. S. 1991 Response of phosphorus and Rhizobium culture on grain yield of lentil. Lens, 18 (1 – 2) : 14 – 19.

Bajpai, P. D. 1965 The influence of selected microflora on solubilisation of phosphorus and uptake of this element by wheat, cowpea and berseem crops. Ph. D. Thesis, P. G. School, I. A. R. I., New Delhi.

Bajpai, P. D. and Sundara Rao 1971 Phosphate solubilizing bacteria. III. Soil inoculation with phosphorus solubilizing bacteria. Soil Sci., Plant Nutrition, 17 (2) : 46 – 53.

Balls, A. K. 1946 Bacterial fertilizers for field crops. Data from Dr. Cho. Seigel Joint Intel. Obi-Agen., Washington D. C. Final report : 494, cited by Allison (1949), Soil Sci., 64 : 413.

Bandopadhyay, S. K. 1988 Variation in host and bacteria in the nodulation of phaseolus species. Environment-and-Ecology, 6(2) : 542 – 546.

Bells, R. W.; Edwards, D. G. and Asher, C. J. 1990 Growth and nodulation of tropical food legumes in dilute solution culture. Plant and Soil, 122 (2) : 249 – 258.

Bordiya, M. C. 1970 Studies on microbiological solubilization of rock phosphate. Ph. D. Thesis, P. G. School, I. A. R. I., New Delhi.

Bdijerink, M. N. 1901 Ulber oligenitrophile mikroben, Zenstr. F. Sakter. AbT., M. 561 cited by Rubenchik : 38

Berezova, E. E.; Naumova, A. N. and Rzanitsyna, E. A. 1938 Mode of action of azotogen. Diklady Akadami, Nauk, S. S. S. R., 18 (6). Cited by Rebenchik : 238.

Bhatnagar, S. S.; Jauhari, K. S. and Iswaran, V. 1979 Associative effective of inoculation of Bacillus megatarium var. phosphaticum and homologus Rhizobium on the yield of *Vigna radiata* and Glycine max. Legume Research, 2 (2) : 106 – 108.

Bhide, V. P. and Purandare, A. G. 1979 Occurrence of Azotobacter within the root cells of cynodon doctylon. Curr. Sci., 48 :913 – 914.

Boyarovich, N. 1954 Bacterial fertilizers – sure means of increasing crops. Sel. Skoe Khozyaistvo Kazakhstana, No. 2.

Brown, M. E.; Burlingham, S. K. and Jackson, R. M. 1962 Studies of Azotobacter species in soil II. Population of Azotobacter in the rhizosphere and effect of artificial inoculation. Plant and Soil, 17 : 320 – 332.

Brown, M. E.; Burlingham, S. K. and Jackson, R. M. 1964 Studies of Azotobacter species in soil III. Effect of artificial inoculation on crop yield. Plant and Soil, 20 : 194 –214.

Burrakova, V. V. 1956 Microflora of soils, in the north yenisei area and ways of increasing their metabolic activity. Author's summary of thesis, Riga. Cited by Rubenchick, P. 215.

Chandra, R. 1991 Influence of different levels of Rhizobium inoculum and pon nodulation. Legume Research.

Cooper, R. 1959 Bacterial fertilizers in the Soviet Union. Soils and Fert., 21 : 327.

Dean, L. A. and Fried, M. 1952 Soils and fertilizers 4. Ed. Pierre, Y. H. and lorman, A. N. Acad. Press, New York. PP 46 – 82.

Dhillon, G. S.; Kler, D. S.; Walia, A. S. and Chanal, V. P. S. 1980 Effect of Azotobacter chroococcum and seed size on the growth and yield of maize. Ind. J. Agron., 25 (2) : 244 – 249.

Doboreiner, J.; Day, J. M. and Dart, P. J. 1973 Fixation of Nitrogen in the rhizosphere of pisum notatum and Sugarcane. Agronomia, 8 : 153 – 157.

Edathil, T. T.; Manian, S. and Udaiyan, K. 1996 Interaction of multiple VAM fungal species of root wionizabion, plant growth and nutrient status of

tomato seedlings. Agri. Ecosystem and Environment, 59 (1/2) : 63–68.

Fernandez – Pascual – M. and Felipe – M R – de 1994 Essentiality of symbiotic de-nitrogen fixation in Pea Rhizobium nodules. Plant Physiol., 104:85–90.

Gaur, A. G.; Madan, M and Ostwal, K. P. 1973 Solubilisation of phosphatic compounds by native microflora or rock phosphate. Ind. J. Expt. Biol., 11 : 427 – 429.

Gaur, A. C. and Negi, A. S. 1980 Bacterial solubilisation of phosphate as evidenced by yield of potato crop. Plant and Soil, in Press. Cited by N. S. Subba Rao.

Greegardt., A. G. 1951 Granular bacterial fertilizers. Agro biologia, No. 5

Gerlach, M. U. and Vogel, J. 1902 Nitrogen fixing bacteria. Centbl. Bakt. Abt., 9 : 817 – 821. Cited by Rubenchik, 269.

Gerresten, F. C. 1948 The influence of micro-organisms on the phosphate intake by the plant. Plant and Soil, 1 (1) : 51.

Gopalakrishnamurthy, A.; Mahadevan, A. and Rangaswami, G. 1967 Effect of bacterization of rice seeds on the rhizosphere microflora and plant growth. Ind. J. Microbiol., 7 (1) : 21.

Goswami, K. P. and Sen, A. 1962 Solubilisation of calcium phosphate by three strains of phosphobacterinum. Ind. J. Agric. Sci., 32 (2) : 76 – 101.

Greeaves, J. E. and Johnes, L. W. 1942 Inoculation of soil with Azotobacter chroococcum. Soil Sci., 83 : 229.

Hegle, D. M. and Dwivedi, B. S. 1994 Crop response to biofertilizers in irrigated areas. fertilizer News, 39 (4) : 19 – 26.

Huser, R. 1965 Plant and Soil. 23 : 236 – 264. Cited by Quispel, P 95.

Jagdale, N. B. 1977 Effect of different doses of Azotobacter inoculum on yield of jowar and Rhizobium inoculation on yield of gram. Mahatma Phule Krishi Vidyapeeth, Rahuri, 413 705 Maharashtra.

Jain, M. K. and Rewari, R. B. 1975 Studies of Azotobacter and Rhizobium inoculation on soyabean. Agriculture Louvain, 23 : 37 – 46. Cited from Soils an Fert., 39 : 49.

Jain, V.; Joshi, J. N. and Taneja, K. D. 1988 Effect of P and Rhizobium on proline content. Agri-Science-Digest-India, 8 (1) : 9 – 11.

Joi, M. B. and Shinde, P. A.	1976	Response of onion crop to Azotobacterization. J. Maharashtra Agric. Univ., 1 :161 – 162.
Karaguishieva, D.	1967	Role to free living nitrogen fixers in accumulation of nitrogen in soils of Alma – Atin region. Vest. Akad. Naukkazakh. SSR., 3 : 52 – 54.
Kasiranjan, C.; Purushothaman, D.; Sankaran, N. and Subramaniam, V.	1976	Usefulness of Azotobacter in economising nitrogenous fertilizers in crop productivity. Proc. National Symposium, Hissar, India. Cited from Soils and Fert., 1977, 40: 205.
Katyal, J. C.; Venkateswarlu, B. and Das, S. K.	1994	Biofertilizers to nutrient supplementation in dry land Agri-Potential and Problems. Fertilizer News, 39 (4) : 27 – 32.
Kholopov. V. D.	1952	The efficiency of Azotobacterin in Western Siberia, Author's summary of thesis, Tomsk.
Khuller, S. and Chahal, V. P. S.	1977	Effect of Azotobacter inoculation on carrot. Ind. J. Microbiol., 17 : 45.
Kirakosyan, A. V.; Petrosyan, A. P. and Azoryan, E. kh.	1949	The effect of bacterial activators on the efficiency of Azotobacter. Microbiologicheskii Sbornik Akademii Nauk Armvanskoi SSR No. 4. Cited by Rubenchik : 215.

Kostichev, S.; Sheloumova, A. and Shulgina, O. 1920 Tr. Otd. S. Kn. Mikrobiol. I. Cited by Mishustin *et al.*, 1962, in Mikrobiol.

Kudzin, yu., K. and Yaroshenko, I. V. 1955 The efficiency of phosphobacterin in chernozem and chestnut soils of the steppezone of Ukranine. Zimledelis No. 4. Cited by Rubenchik : 214.

Kudzin, yu., K. and Yaroshenko, I. V. 1962 Use of phosphobacterin in the Chernozem zone. Mikrobiologiya, 31 : 1098 – 1101.

Kulikovs'ka, M. D. 1954 The effect of Azotobacter on the dynamics of nitrogen in soil. Mikrobiologicheskii Zhurnal Akademii Nauk. Ukrainskei SSR 16. Cited by Rubenchik : 220.

Kulikovs'ka, M. D. 1955 The effect of Azotobacter on the accumulation of nitrogen in soil and on some groups of soil micro-organisms. Author's summary of Thesis. Kieve. Cited by Rubenchik : 220.

Kumar, P. and Agarwal, J. P. 1993 Response of lentil to Rhizobium inoculation, N and P fertilization. Indian j. of Agronomy, 38 (2), 318 – 320.

Kumar and Mangal 1997 Effect of VAM fungi and phosphorus level on dry weight and mineral composition of onion. Haryana J. Hort. Sci., 26 (3-4) : 250 – 254.

Kundu, B. S. and Gaur, A. C. 1981 Inoculant carriers for phosphate solubilising micro-organisms. Ind. J. Agric. Sci., 51 (4) : 252 – 255.

Kvasnikov, E. I. 1958 Ecological principles of the use of bacterial fertilizers in Central Asia. 'Izdated' Stvo Akademii Nauk Ukrainskei SSR, Kiev. Cited by Rubenchik : 214.

Lakshmi Kumari, M.; Vijayalakshmi, K. and Subbarao, N. S. 1972 Interaction between Azotobacter sp. and fungi. I. In Vitro studies with Fusarium moniliforme sheld. Phytopath. 2, 75 : 27 – 30.

Laura, R. D.;Roy, M.; Sangwan, P. S. and Bagga, R.K. 1994 Effect of inorganic, organic and biofertilizer on permitted yields in dryland areas. Crop Research, Hissar, 8 (3) : 499 – 503.

Linchevskaya, M. P. and Kaliberda, V. M. 1958 The effect of bacterial fertilizers on potato crops Izatel Stvo Akademii Nauk. Ukrainskoi SSR, Kiev. Cited by Rubenchik : 214.

Mauna, J. 1966 Ann. Inst. Pasteur (Suppl. No. 3), 111 : 9 – 38. Cited by Quispel : 98.

Mahmoud, S. A. Z.; El-Sawy, M.; Ishac, Y. Z. and El-Safty, M. M. 1978 The effect of salinity and alkalinity on the distribution and capacity of N_2 fixation by Azotobacter in Egyptian soils. In : Environmental role of Nitrogen fixing Blue-green Algae and Asymbiotic bacteria, Ecological Bulletins (Stockholm), 26 : 99 – 109. Cited from Soils and Fert., 42 : 690.

Manil, P. and Brakel, J. 1965 Azotobacter and rhizosphere. Some trials made in Belgium. C. R. Seane Soc. Biol., 159 : 1025 – 1027. Cited from Soil and Fert., 31 : 683.

Marinova, R. 1970 Effect of Mineral fertilizers and micronutrients on soyabean seed yield and quality. CF. F.C.A. 25 (1) : 81.

Mourya, B. R. and Sonoria, C. L. 1986 Beneficial effects of co-inoculating chick pea seed with Rhizobium, Azotobacterin and pseudomonas. Indian Jou. of Agri. Sci., Vol. 56 (6) : 463 – 466.

Mehrotra, C. L. and Lehri, L. K. 1971 Effect of Azotobacter inoculation on crop yield. J. Indian Soc. Soil Sci., 19 : 243 – 248.

Milosevic, N.; Govindonca, M.; Jarak, M.; Bogdonic, B. and Ubovic, M. 1995 Number of micro-organisms and dehydroginase activity in soil under Peas, Onion and Cabbage. Microbiologic (Yugoslavia) 32 (2) : 259 – 267.

Minasyan, A. I. and Nalbandyan, A. D. 1965 Effect of Azotobacterin on rooting and growth of vine cuttings. Dokl. Akad. Nauarnyn S.S.R., 41 : 251–255. Cited from Soil & Fert., 1968, 31 : 683.

Mishustin, E. N. 1970 The importance of non-symbiotic nitrogen fixing micro-organisms in agriculture. Plant and Soil. 32 : 545 – 554.

Mitushtin, E. N. and Naumova, A. N. 1956 The use of bacterial fertilizers in sowing vegetable seeds into Peat Manure nutrient cubes. Mikrobiologia, 25 : 41 – 48.

Mishustin, E. N. and Naumova, A. N. 1962 Bacterial fertilizers, their effectiveness and mode of action. Microbiol., 31 : 442.

Mitchel, J. 1953 Crop variety response to applied phosphate and uptake of phosphorus and uptake of phosphorus from soil and fertilizers. Agron. J., 45:1–11.

Nair, K. S.; Ramaswamy, P. P. and Perumal, R. 1972 Studies of Azotobacter IV. Effect of Azotobacter inoculation on paddy. Madras Agril. J. 59 : 28 – 30.

Naumova, A. N. 1939 The effect of seed bacterization on the susceptibility of spring wheat seedling to fungal infections on crops. Mikrobiologiya, 8 (2). Cited by Rubenchik : 228.

Nenec., A. and Pecina, L. 1963 Experiments on the application of Azotobacter preparations in the bacterial inoculation of potato. Ust. Ved. Inf. MZLVH (Rostl. Vyroba) 36 : 715 – 727.

Oblisami, G.; Natarajan, T. and Balaraman, K. 1976 Response of sunflower to Azotobacter inoculation. Indian J. Agron., 22 : 250.

Patel, J. J. 1969 Micro-organisms in the rhizosphere of plants inoculated with Azotobacter chroococcum. Plant and Soil, 31 : 209 – 223.

Patil, S. P.; Patil, P. L. and More, B. B. 1976 Effect of association of Azotobacter with nitrogenous fertilizer on yield of paddy. Madras Agric. J., 63 : 600 – 602.

Paul, N. B. 1966 Physiology of phosphorus solubilising microflora and mechanism of phosphorus solubilisation. Ph. D. Thesis, P. G. School, I. A. R. I., New Delhi

Paul, E. A.; Myers, E. K. J. and Rice, W. A. 1971 Plant and Soil, sp. Vol.; 495 – 507. Cited by Quispel, A. P 99.

Pelczar, M. J. and Reid, R. D. 1958 Microbiology. McGraw Hills.

Pikovskaya, R. I. 1948 Mobilisation of phosphate in soil in connection with the vital activity of some microbial species. Microbiologiya, 17 : 362 – 370. Cited from Soils & Fert., 12 : 347.

Pisenskaya, V. A. and Kochienova, T. A. 1956 The use of bacterial fertilizers for winter wheat under the arid conditions of the stravrpool border. Byull Mauchtekh. Inform staviol. Mauch. issled. Inst. Sel. Khoz No. 1 – 2 : 52 – 53. Cited from Soils and Fert., 12 : 941.

Prathiban, S. and Thamburaj, S. 1991 Influence of Rhizobium culture and nitrogen fertilization on French beans. South Indian Horticulture, 36 (3) : 137 – 138.

Rana, D. S.; Meelu, O. P.; Sharma, K. N. and Randhawa, N. S. 1975 Effect of phosphobacteria on the availability of phosphorus from rock phosphate. J. Res. P. A. U., Ludhiana, 123 (3) : 232 – 238.

Rangarajan, M. and Muthukrishan, P. 1976 Effect of various organic manures and Azotobacter chroococcum on the grówth and yield of paddy. Madras Agric. J., 63 : 611 – 615.

Rao, A. V. and Sharma, R. L. 1981 Effect of Azotobacter on wheat and tomato. Indian J. Microbiol., 21 (1) : 25 – 27.

Rim – Yong – dal. 1992 Study on the characteristics and effect of symbiotic nitrogen fixing Azotobacterin, Acta of academy of Agri. Sci. (NO_3): 61 – 66.

Riozin, M. B. and Ezrukh, E. N. 1958 The use of bacterial fertilizers in polar agricultural conditions. Tzdatel Stvo Akademii Nauk Ukrainskai S.S.R., Cited by Rubenchik : 43.

Rosario, Azoon and Barea, J. M. 1975 Synthesis of Auxins, Gibberellins and cytokinisis by Azotobacter vine landi and Azotobacter beijerinekii related to effects produced on tomato plants. Plant and Soil, 43 : 609 – 619.

Rovira, A. D. 1963 Microbial inoculation of plants I. Establishment of free living nitrogen fixing bacteria in the rhizosphere and their effects on Maize, Tomato and Wheat. Plant and Soil, 19 : 304 – 314.

Roy, S. K. and Rahaman, S. M. L. 1992 Effect of seed rate and inoculation on nodulation, growth and yield of lentil. Legume Research, 15 (3–4) : 131 – 136.

Rubenchik, L. I. 1941 Inter-relationship between Azotobacter and some other soil bacteria. All union conference on Agricultural Microbiology. Microbiol., 10. Cited by Rubenchik :151.

Rubenchik, L. I. 1963 "Azotobacter and its use in Agriculture" (Nat. Sci. Found., Washington D.C. and Israel Programme for Scientific translocations Ltds.) : P 268 .

Rubenchik, L. I.; Kil Cherskay, A. A. and Kardyum, V. A. 1958 Inter-relationship between Azotobacter and Bacillus megatarium. var. phosphaticum Sbonik. Akademii, Nauk Ukrainskai S.S.R. Cited by Rubenchik : 148.

Saber, M. S. M. and Kabesh, M. O. 1990 Application on yield and nutrient uptake by lentil plants, 30 (3) : 415 – 422.

Sandrak, N. A. 1971 Effect of extra cellular secretions of Azotobacter on other micro-organisms. Mikrobiologiya, 40: 691 – 695.

Sanoria, C. L. and Sundara Rao, W. V. B. 1973–74 Growth promoting effect of different strains of Azotobacter chroococcum on jowar. J. Scientific Research, B. H. U, XXIV :140 – 142.

Seigal, O. and Schmidt., H. L. 1966 Laboratory experiments and results of field trials on inoculation with Azotobacter, Land W. Forsch., 19 : 18 – 28.

Sen, A. and Paul, N. N. 1957 Solubilisation of phosphorus by some common soil bacteria. Curr. Sci., 26 : 222.

Shah, K. M. 1959 Phosphobacterin inoculation of berseem seed and its effects on phosphorus availability of soil and fertilizer phosphorus M. Sc. (Ag.) Thesis, Div. Agron. I. A. R. I., New Delhi.

Sharma, R. L. and Rao, A. V. 1978 Introduction of Azotobacter with Rhizobium in Soyabean rhizosphere. Fert. Technol. 15 : 50 – 51.

Sharma, J. P. and Singh, M. 1971 Effect of phosphobacterin culture on the efficiency of phosphate fertilizers and on the yield of maize. Indian J. Agron., 16 (3): 422 – 424.

Sheloumova, A. M. 1941 The mode of action of azotozen. Mikrobiologiya, 10. Cited by Rubenchik : 215.

Shestakova 1963 Use of phosphobacterin for improving seedling growth. Lesn. Khoz., 16 (1) : 32–35. Cited from Soils and Fert., 28 (2481) Year 1965.

Sidorova, K. K.; Simakov, G. A. and Stolyarov, S. N. 1990 Evaluation of pea varieties for nitrogen fixing activity. Sibirski-Vestnik-Selskokhoz Yanistvennai-Nauki, No. 2 : 28 – 34.

Sinha, S. B. 1958 Contribution of Azotobacter in nitrogen fixation under natural conditions. J. Indian Soc. Soil Sci., 6 : 153 – 159.

Singh, R. V. and Singh, R. R. 1989 Effect of levels of N, P and seeding rates in Rhizobium inoculated seed of cluster bean. Annals of Agri. Research, 13 (3) : 229 – 306.

Singh, R. V. and Singh, R. R. 1990 Uptake of N, P and K by guar as influenced by nitrogen (with and without seed inoculation). Annals of Agri. Research, 11 (3) : 329 – 332.

Smith, J. H. and Allison, F. E. 1962 Phosphobacterin as a soil inoculant Laboratory. Greenhouse and fields evaluation, USDA economics. Bull., 1263 : 22.

Smith, J. H.; Allison, F. E. and Sonlides 1961 Evaluation of phosphobacterin as a soil inoculant. S. S. A. C. Prod., 25 : 109.

Sperber, J. I. 1957 Solution of mineral phosphate by soil bacteria. Nature, Lord, 180 : 994 – 995.

Steward, A. B. and Williams, E. G. 1942 Time of application of fertilizer with special reference to super phosphate. Scot. J. Agric., 24:52–56.

Streuber, E. 1967 Problem of inoculation and nitrogen fertilizing of soyabeans. Zentbl. Bakt. parasitkole, 121, Abt. 11 : 709 – 718. Cited from Soils and Fert., 33 : 132.

Subba Rao, N. S. 1977 "Soil micro-organisms and plant growth". Oxford and IBH Publishing Co.

Subba Rao, N. S. and Bajpai, P. D. 1965 Fungi on the surface of legume root nodules and phosphate solubilisation. Experientia, 29 : 386 – 387.

Sulaiman, M. 1971 Non symbiotic nitrogen fixation in rice plants. Beitr. Trop. Subtrop. Landwiri Tropenveterinormed 9: 139 – 146. Cited by Subba Rao, N. S. in : Recent advances in biological N_2-fixation, P 211.

Sundara Rao, W. V. B. and Iswaran, V. 1959 Nitrogen fixation by Azotobacter chroococcum in culture. J. Indian Soc. Soil Sci., 7 : 91.

Sundara Rao, W. V. B.; Mann, H. S.; Paul, N. B. and Rathaur, S. P. 1963 Bacterial inoculation experiments with special reference to Azotobacter. Indian J. Agric. Sci., 279 – 290.

Sundara Rao, W. V. B. and Sinha, M. K.	1963	Phosphate dissolving micro-organisms in the soil and rhizosphere. Indian J. Agric. Sci., 33 (4) : 272 – 278.
Swaby, R. J. and Joan Sporber	1958	Phosphorus dissolving micro-organisms in rhizosphere of legumes. Nutrition of legumes. Proc. Univ. Nottingham, 5th Easter School Agric. Scientist : 289.
Taha, S. M.; Mohaamad, S.A,C,; El-Damaty, A. H. and Ab. El Hafez, A. M.	1969	Activity of phosphate dissolving bacteria in Egyptian soils. Plants and Soil, 31 (1) : 149 – 160.
Tohan, V. T. and Jackson, D. L.	1966	Studies of nitrogen fixing bacteria IX. Study of inoculation of wheat with Azotobacter in laboratory and field experiments. Proc. linn. Soc. N. S. V., 90 : 290 – 298.
Teplvakova, Z. F.	1955	The effect of bacterial fertilizers in Kuzakhstan, Vestnik Akademii Nauk Kazakhskoi S.S.R., 10 : 127.
Terry,E.; Pino.DE.; Los, A. and Medina, N.	1995	Biofertilizer application in early season tomato cultivates and tropicales, 16 (3) : 69 – 71.
Thompson, J. P.	1974	Seed inoculation of wheat and barley with Azotobacter chroococcum in Queensland. Od. J. Agric.

Amm. Sci., 13 : 129 – 138.

Truffant, G. and Eezssonoff, N. 1924 Comp. Rend. Soc. Bid. Cited from Allison (1947).

Tzchapek, M. and Garbosky, A. J. 1952 New concepts of the activity of Azotobacter in soil. India, 5 : 12 – 16. Cited from Soils and Fert. (1953), 16 : 561.

Vakshnil and Nanorik 1954 The enrichment of dung and the use of biologically enriched compost. Agrobiologia, 3 (24–33). Cited from Soils and Fert., 17 (2475).

Verma, T. S.; Thakur, P. C. and Singh, A. 1997 Effect of biofertilizer on vegetable and seed yield of cabbage.

Vigileva, A. I. 1954 Mixed cultures of Azotobacter and root nodule bacteria and their use in agriculture. Author's summary of Thesis Moskova. Cited by Rubenchik : 215.

Volodin, V. 1969 Effect of treating seeds potatoes with Mo and Azotobacterin. Kartofel, Ovoshchi, 6 : 46. Cited from Soils and Fert., 35 : 69.

Vorhees, E. B. and Lipman, J. G. 1907 A review of investigation in soil microbiology. U. S. Dept. Agri off Exp. Sta. Bul., 194 : 1 – 108.

Wange, S. S.; Patil, P. L.; Meher, B. B. and Karkeli, M. S. 1996 Response of cabbage to microbial inoculants and incremental levels of nitrogen. Journal of Maharashtra Agri. Univ., 20 (3) : 429 – 430.

Yanni, Y. G. 1992 Performance of chickpea, lentil and lupin nodulated with indigenous or inoculated rhizobia. World Journal of Microbiol. and Biotech., 8 (b) : 607 – 613.

Yousry, M.; Kabesh. M. O. and Saber, M. S. M. 1978 Manganese availability in a calcerous soil as a result of phosphate fertilization and inoculation with phosphobacterin. African J. Agril. Sci., 5 (2) : 75 – 80.

Young, J. A. 1954 The effectiveness of phosphobacterin on sod podsolized soils of the Kirov region. Ze'mledelie, (10):62–70. Cited from Soils and Fert.,18 (297).

Yuki, M. M.; Machado, J. O.; Cherata Masca, M. C. G. and Andriali, J. L. 1994 Response of tomato plant to inoculation with rhizosphere soil of Paspalum Novatatum VAM fungi and Azotobacterin paspali. Cientifica – (Jaboticabal), 22 (1) : 53 – 62.

APPENDIX

1 **Plant height (cm)** **Wheat 30 DAS 2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.23	0.11	1.20	3.740	**NS**
Due to Treatments	7	212.61	30.37	319.11	2.775	**S**
Due to Error	14	1.33	0.10	-	-	
Total (rt-1)	23	214.16	-	-	-	

2 **Plant height (cm)** **Wheat 30 DAS 2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.21	0.10	0.53	3.740	**NS**
Due to Treatments	7	212.21	30.32	154.85	2.775	**S**
Due to Error	14	2.74	0.20	-	-	
Total (rt-1)	23	215.15	-	-	-	

3 **Plant height (cm)** **Wheat 60 DAS 2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	2.52	1.26	6.52	3.740	S
Due to Treatments	7	816.51	116.64	603.52	2.775	S
Due to Error	14	2.71	0.19	-	-	
Total (rt-1)	23	821.73	-	-	-	

4 **Plant height (cm)** **Wheat 60 DAS 2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	5.36	2.68	8.03	3.740	S
Due to Treatments	7	831.66	118.81	355.98	2.775	S
Due to Error	14	4.67	0.33	-	-	
Total (rt-1)	23	841.70	-	-	-	

5 **Plant height (cm)** **Wheat 90 DAS 2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	1.27	0.63	3.40	3.740	NS
Due to Treatments	7	766.55	109.51	588.34	2.775	S
Due to Error	14	2.61	0.19	-	-	
Total (rt-1)	23	770.43	-	-	-	

6 **Plant height (cm)** **Wheat 90 DAS 2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.41	0.20	0.63	3.740	NS
Due to Treatments	7	774.62	110.66	342.82	2.775	S
Due to Error	14	4.52	0.32	-	-	
Total (rt-1)	23	779.55	-	-	-	

7 **Grain yield (q/ha)** **Wheat** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.27	0.14	0.56	3.740	**NS**
Due to Treatments	7	273.57	39.08	161.12	2.775	**S**
Due to Error	14	3.40	0.24	-	-	
Total (rt-1)	23	277.24	-	-	-	

8 **Grain yield (q/ha)** **Wheat** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.77	0.38	1.54	3.740	**NS**
Due to Treatments	7	276.99	39.57	159.15	2.775	**S**
Due to Error	14	3.48	0.25	-	-	
Total (rt-1)	23	281.23	-	-	-	

9 **Straw in wheat (q/ha)** **Wheat** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	1.25	0.63	1.74	3.740	**NS**
Due to Treatments	7	540.42	77.20	215.20	2.775	**S**
Due to Error	14	5.02	0.36	-	-	
Total (rt-1)	23	546.69	-	-	-	

10 **Straw in wheat (q/ha)** **Wheat** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	2.37	1.18	1.53	3.740	**NS**
Due to Treatments	7	612.58	87.51	113.33	2.775	**S**
Due to Error	14	10.81	0.77	-	-	
Total (rt-1)	23	625.76	-	-	-	

11 **Grain - Straw ratio in wheat** **Wheat** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.0058	0.0029	0.43	3.740	**NS**
Due to Treatments	7	0.17	0.02	3.68	2.775	**S**
Due to Error	14	0.09	0.01	-	-	
Total (rt-1)	23	0.27	-	-	-	

12 **Grain - Straw ratio in wheat** **Wheat** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.00152	0.00076	1.42	3.740	**NS**
Due to Treatments	7	0.15	0.02	41.24	2.775	**S**
Due to Error	14	0.01	0.00	-	-	
Total (rt-1)	23	0.16	-	-	-	

13 **Nitrogen uptake in wheat grain (kg/ha)** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.000012	0.000006	0.26	3.740	**NS**
Due to Treatments	7	0.496856	0.070979	3025.77	2.775	**S**
Due to Error	14	0.000328	0.000023	-	-	
Total (rt-1)	23	0.497196	-	-	-	

14 **Nitrogen uptake in wheat grain (kg/ha)** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.00010	0.00005	1.06	3.740	**NS**
Due to Treatments	7	0.48388	0.06913	1538.36	2.775	**S**
Due to Error	14	0.00063	0.00004	-	-	
Total (rt-1)	23	0.48460	-	-	-	

15 **Phosphorus uptake in wheat (kg/ha)** **Wheat** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.000004	0.000002	1.60	3.740	**NS**
Due to Treatments	7	0.000560	0.000080	62.49	2.775	**S**
Due to Error	14	0.000018	0.000001	-	-	
Total (rt-1)	23	0.000582	-	-	-	

16 **Phosphorus uptake in wheat (kg/ha) Wheat 2001 - 200**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.000005	0.0000025	3.26	3.740	**NS**
Due to Treatments	7	0.00053	0.00008	98.29	2.775	**S**
Due to Error	14	0.000011	0.0000008	-	-	
Total (rt-1)	23	0.00055	-	-	-	

17 **Nitrogen and phosphorus uptake ratio in wheat 2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.04	0.02	0.19	3.740	**NS**
Due to Treatments	7	339.59	48.51	463.34	2.775	**S**
Due to Error	14	1.47	0.10	-	-	
Total (rt-1)	23	341.09	-	-	-	

18 **Nitrogen and phosphorus uptake ratio in wheat 2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	7.03	3.52	2.29	3.740	**NS**
Due to Treatments	7	336.89	48.13	31.31	2.775	**S**
Due to Error	14	21.52	1.54	-	-	
Total (rt-1)	23	365.44	-	-	-	

19 **Nitrogen uptake in wheat straw (kg/ha)** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.00037	0.00018	7.04	3.740	S
Due to Treatments	7	0.06901	0.00986	380.33	2.775	S
Due to Error	14	0.00036	0.00003	-	-	
Total (rt-1)	23	0.06974	-	-	-	

20 **Nitrogen uptake in wheat straw (kg/ha)** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.00037	0.00018	5.75	3.740	S
Due to Treatments	7	0.06988	0.00998	312.01	2.775	S
Due to Error	14	0.00045	0.00003	-	-	
Total (rt-1)	23	0.07069	-	-	-	

21 **Phosphorus uptake in wheat straw (kg/ha)** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.0000011	0.0000005	0.92	3.740	NS
Due to Treatments	7	0.00063	0.00009	153.82	2.775	S
Due to Error	14	0.00001	0.00000	-	-	
Total (rt-1)	23	0.00064	-	-	-	

22 **Phosphorus uptake in wheat straw (kg/ha)** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.00001	0.0000039	6.04	3.740	S
Due to Treatments	7	0.00065	0.00009	144.53	2.775	S
Due to Error	14	0.00001	0.0000006	-	-	
Total (rt-1)	23	0.00067	-	-	-	

23 **Nitrogen and Phosphorus uptake ratio in wheat straw** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.18	0.09	9.17	3.740	S
Due to Treatments	7	3.38	0.48	48.37	2.775	S
Due to Error	14	0.14	0.01	-	-	
Total (rt-1)	23	3.70	-	-	-	

24 **Nitrogen and Phosphorus uptake ratio in wheat straw** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.000033	0.000017	1.00	3.740	NS
Due to Treatments	7	4.02	0.57	34429.11	2.775	S
Due to Error	14	0.000233	0.000017	-	-	
Total (rt-1)	23	4.02	-	-	-	

1 **Plant height (cm)** **Pea** **30 DAS** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.26	0.13	6.95	3.740	**S**
Due to Treatments	7	22.61	3.23	175.58	2.775	**S**
Due to Error	14	0.26	0.02	-	-	
Total (rt-1)	23	23.12	-	-	-	

2 **Plant height (cm)** **Pea** **30 DAS** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.36	0.18	1.41	3.740	**NS**
Due to Treatments	7	20.99	3.00	23.33	2.775	**S**
Due to Error	14	1.80	0.13	-	-	
Total (rt-1)	23	23.15	-	-	-	

3 **Plant height (cm)** **Pea** **60 DAS** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.99	0.49	5.75	3.740	**S**
Due to Treatments	7	36.77	5.25	61.25	2.775	**S**
Due to Error	14	1.20	0.09	-	-	
Total (rt-1)	23	38.96	-	-	-	

4 **Plant height (cm)** **Pea** **60 DAS** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	3.47	1.73	4.51	3.740	**S**
Due to Treatments	7	41.25	5.89	15.34	2.775	**S**
Due to Error	14	5.38	0.38	-	-	
Total (rt-1)	23	50.10	-	-	-	

5 **No. of pods per plant** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.0025	0.0012	0.16	3.740	**NS**
Due to Treatments	7	2.00	0.29	36.14	2.775	**S**
Due to Error	14	0.11	0.01	-	-	
Total (rt-1)	23	2.12	-	-	-	

6 **No. of pods per plant** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.61	0.30	4.10	3.740	S
Due to Treatments	7	1.75	0.25	3.38	2.775	S
Due to Error	14	1.03	0.07	-	-	
Total (rt-1)	23	3.39	-	-	-	

7 **Length of pod (cm)** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.05	0.03	0.80	3.740	**NS**
Due to Treatments	7	3.57	0.51	15.30	2.775	**S**
Due to Error	14	0.47	0.03	-	-	
Total (rt-1)	23	4.09	-	-	-	

8 **Length of pod (cm)** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.11	0.05	0.33	3.740	**NS**
Due to Treatments	7	4.83	0.69	4.25	2.775	**S**
Due to Error	14	2.27	0.16	-	-	
Total (rt-1)	23	7.21	-	-	-	

9 **Number of grains per pod** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.75	0.38	1.62	3.740	**NS**
Due to Treatments	7	8.00	1.14	4.92	2.775	**S**
Due to Error	14	3.25	0.23	-	-	
Total (rt-1)	23	12.00	-	-	-	

10 **Number of grains per pod** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.58	0.29	0.47	3.740	**NS**
Due to Treatments	7	6.63	0.95	1.51	2.775	**NS**
Due to Error	14	8.75	0.63	-	-	
Total (rt-1)	23	15.96	-	-	-	

11 **Number of nodules** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.60	0.30	1.68	3.740	**NS**
Due to Treatments	7	851.35	121.62	679.95	2.775	**S**
Due to Error	14	2.50	0.18	-	-	
Total (rt-1)	23	854.46	-	-	-	

12 **Number of nodules** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	2.23	1.11	2.47	3.740	**NS**
Due to Treatments	7	836.97	119.57	265.28	2.775	**S**
Due to Error	14	6.31	0.45	-	-	
Total (rt-1)	23	845.50	-	-	-	

13 **Grain yield (q/ha)** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	1.05	0.53	0.89	3.740	**NS**
Due to Treatments	7	1190.90	170.13	288.50	2.775	**S**
Due to Error	14	8.26	0.59	-	-	
Total (rt-1)	23	1200.21	-	-	-	

14 **Grain yield (q/ha)** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	1.21	0.60	0.97	3.740	**NS**
Due to Treatments	7	1168.26	166.89	268.75	2.775	**S**
Due to Error	14	8.69	0.62	-	-	
Total (rt-1)	23	1178.16	-	-	-	

15 **Fresh weight of leaf (g)** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.97	0.48	5.78	3.740	**S**
Due to Treatments	7	1387.96	198.28	2367.52	2.775	**S**
Due to Error	14	1.17	0.08	-	-	
Total (rt-1)	23	1390.10	-	-	-	

16 **Fresh weight of leaf (g)** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.02	0.01	0.01	3.740	**NS**
Due to Treatments	7	1461.75	208.82	245.04	2.775	**S**
Due to Error	14	11.93	0.85	-	-	
Total (rt-1)	23	1473.70	-	-	-	

17 **Fresh weight of shoot (g)** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.27	0.13	0.47	3.740	**NS**
Due to Treatments	7	2405.99	343.71	1217.77	2.775	**S**
Due to Error	14	3.95	0.28	-	-	
Total (rt-1)	23	2410.21	-	-	-	

18 **Fresh weight of shoot (g)** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.83	0.42	0.56	3.740	**NS**
Due to Treatments	7	2358.28	336.90	450.30	2.775	**S**
Due to Error	14	10.47	0.75	-	-	
Total (rt-1)	23	2369.59	-	-	-	

19 **Fresh weight of Root (g)** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.02	0.01	0.81	3.740	**NS**
Due to Treatments	7	5.62	0.80	62.73	2.775	**S**
Due to Error	14	0.18	0.01	-	-	
Total (rt-1)	23	5.82	-	-	-	

20 **Fresh weight of Root (g)** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.10	0.05	0.73	3.740	**NS**
Due to Treatments	7	6.13	0.88	13.11	2.775	**S**
Due to Error	14	0.94	0.07	-	-	
Total (rt-1)	23	7.17	-	-	-	

21 **Dry weight of Leaf (g)** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.02	0.01	0.13	3.740	**NS**
Due to Treatments	7	145.38	20.77	317.49	2.775	**S**
Due to Error	14	0.92	0.07	-	-	
Total (rt-1)	23	146.32	-	-	-	

22 **Dry weight of Leaf (g)** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.21	0.10	0.46	3.740	**NS**
Due to Treatments	7	142.95	20.42	92.20	2.775	**S**
Due to Error	14	3.10	0.22	-	-	
Total (rt-1)	23	146.26	-	-	-	

23 **Dry weight of Shoot (g)** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.63	0.31	2.01	3.740	**NS**
Due to Treatments	7	50.23	7.18	46.07	2.775	**S**
Due to Error	14	2.18	0.16	-	-	
Total (rt-1)	23	53.04	-	-	-	

24 **Dry weight of Shoot (g)** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.40	0.20	0.61	3.740	**NS**
Due to Treatments	7	48.88	6.98	21.39	2.775	**S**
Due to Error	14	4.57	0.33	-	-	
Total (rt-1)	23	53.85	-	-	-	

25 **Dry weight of Root (g)** **Pea** **2000 - 2001**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.0034	0.0017	2.78	3.740	**NS**
Due to Treatments	7	1.23	0.18	290.64	2.775	**S**
Due to Error	14	0.01	0.00	-	-	
Total (rt-1)	23	1.24	-	-	-	

26 **Dry weight of Root (g)** **Pea** **2001 - 2002**

Source of Variation	d. f.	S. S.	M. S. S.	Cal. F	Tab. F (5%)	Result
Due to Replications	2	0.03	0.01	0.84	3.740	**NS**
Due to Treatments	7	1.33	0.19	11.63	2.775	**S**
Due to Error	14	0.23	0.02	-	-	
Total (rt-1)	23	1.58	-	-	-	